| OF |

AD
A070240

LEVEL

END
DATE
FILMED
7-79
DDC

MICROCOPY RESOLUTION TEST CHART
NATIONAL BUREAU OF STANDARDS 1963 A

LEVEL III
A054 658
AD A070240

STUDIES OF METABOLISM, FUNCTION AND MECHANISM

OF DESTRUCTION OF RED CELLS

Final Report

April    1978

( for the period 1 July 1973 to 31 March 1978 )


by


Robert I. Weed, M. D.
Paul L. La Celle, M. D.
Marshall A. Lichtman, M. D.
Jules Cohen, M. D.


Supported by

University of Rochester School of Medicine and Dentistry
Rochester, New York  14642

D D C
RECEIVED
JUN 20 1979
D

DOD DISTRIBUTION STATEMENT

79  06  18  103

# DISCLAIMER NOTICE

THIS DOCUMENT IS BEST QUALITY
PRACTICABLE. THE COPY FURNISHED
TO DDC CONTAINED A SIGNIFICANT
NUMBER OF PAGES WHICH DO NOT
REPRODUCE LEGIBLY.

| REPORT DOCUMENTATION PAGE | | READ INSTRUCTIONS BEFORE COMPLETING FORM |
|---|---|---|
| 1. REPORT NUMBER | 2. GOVT ACCESSION NO. | 3. RECIPIENT'S CATALOG NUMBER |
| **4. TITLE (and Subtitle)** <br> Studies of Metabolism, Function and Mechanism of Destruction of Red Cells. | | **5. TYPE OF REPORT & PERIOD COVERED** <br> Final Report. <br> 1 July 1973 - 31 March 1978 <br> **6. PERFORMING ORG. REPORT NUMBER** |
| **7. AUTHOR(s)** <br> Marshall A. Lichtman, M. D. Robert I. Weed, Paul L. LaCelle Jules Cohen | | **8. CONTRACT OR GRANT NUMBER(s)** <br> DADA 17-73-C-3135 |
| **9. PERFORMING ORGANIZATION NAME AND ADDRESS** <br> University of Rochester School of Med.& Dent. <br> Rochester, New York 14642 | | **10. PROGRAM ELEMENT, PROJECT, TASK AREA & WORK UNIT NUMBERS** <br> 62772A <br> 3S762772A814 00 072 |
| **11. CONTROLLING OFFICE NAME AND ADDRESS** <br> US Army Medical Research and Development Cmd. <br> Fort Detrick, Frederick, Maryland 21701 | | **12. REPORT DATE** <br> April 1978 <br> **13. NUMBER OF PAGES** <br> 44 |
| **14. MONITORING AGENCY NAME & ADDRESS(If different from Controlling Office)** <br> 44 p. | | **15. SECURITY CLASS. (of this report)** <br> Unclassified <br> **15a. DECLASSIFICATION/DOWNGRADING SCHEDULE** |

**16. DISTRIBUTION STATEMENT (of this Report)**

Approved for public release; distribution unlimited.

**17. DISTRIBUTION STATEMENT (of the abstract entered in Block 20, if different from Report)**

**18. SUPPLEMENTARY NOTES**

**19. KEY WORDS (Continue on reverse side if necessary and identify by block number)**

hemoglobin, red cells, oxygen transport, 2,3-DPG

**20. ABSTRACT (Continue on reverse side if necessary and identify by block number)**

We have studied the effects of propranolol on hemoglobin-oxygen affinity and red cell shape, both in vitro and in vivo. We have also examined the effects of hemodialysis on red cell organic phosphates and on oxygen-binding to hemoglobin. Studies were conducted on the role of blood pH alterations in the elevation of red cell 2,3-DPG and decrease in hemoglobin-oxygen affinity observed in subjects with hypoproliferative anemia. Studies of the interactions

DD FORM 1473 EDITION OF 1 NOV 65 IS OBSOLETE LB

UNCLASSIFIED 307 300

of anemia, red cell ATP concentration and changes in plasma inorganic phosphate have been conducted. The interrelationships of red cell magnesium concentration and ATP concentration on hemoglobin oxygen binding have been studied. Studies of the role of red cell aging on flow in capillaries have been performed. We have studied patients with acute myocardial infarction and have found that whole blood oxygen consumption remains constant despite a nearly three-fold variation in arterial oxygen flow rate. We calculated that a reduction in hemoglobin oxygen affinity could explain about one-third of the increased extraction of oxygen required to maintain oxygen consumption. Other, probably, tissue compensating mechanisms must account for the remaining adaptation. We have studied water soluble radiographic contrast materials and have found them to produce a significant alteration in the distribution of irons across the red cell membrane. The normal negative potential of the red cell membrane can be reduced or reversed, depending on the concentration of contrast material added to blood. As the inside of the cell becomes more positive with respect to the outside, protons are in effect repelled into plasma, producing acidemia. These changes also lead to a factitious alteration in hemoglobin oxygen affinity when studied in vitro. We have developed a technique which uses a single venous blood sample to measure the $P_{50}$ or oxygen-hemoglobin affinity state in a patient. These simple techniques allow screening of patients with unexplained polycythemia or anemia for hemoglobins with altered affinity. It has made more accessible and simplified this test. We have also studied the role of hemoglobin oxygen affinity in oxygen transport to ischemic myocardium. This was done in 14 patients undergoing coronary angiography. We have also studied the quantitative role of hemoglobin oxygen affinity in the oxygen transport in patients with chronic congestive heart failure. We have also developed an isolated canine gracilis muscle model to test the hypothesis that altered hemoglobin-oxygen binding can or cannot influence tissue oxygen uptake when either blood flow or arterial oxygen content are held constant.

Accession For

NTIS GRA&I ☒
DDC TAB ☐
Unannounced ☐
Justification_____

By_____

Distribution/

Availability Codes

| Dist | Avail and/or special |

A 23 010

Progress Report for Contract No. DA 17-73-C-3135  July 1, 1973 to March 31, 1978

    1.  It has been found that propranolol decreases the affinity of hemoglobin (hgb) for oxygen ($O_2$) when added to red cell suspensions in vitro by a process which requires an intact cell.  Subsequent studies suggested its effect was produced by a redistribution of membrane-bound 2,3-diphosphoglycerate (2,3-DPG); however, we and others have been unable to find an increased 2,3-DPG/hgb molar ratio in red cell membranes.  Hence, we have re-examined the effect of propranolol on the red cell to further clarify its mechanism of action.

    DL or D-propranolol produces a maximal decrease in hgb-$O_2$ affinity ($P_{50}$=29.5 mmHg, control $P_{50}$=25.5 mmHg) at 0.1mM in buffer or 0.5mM in plasma.  The dose response curve was steep, since 0.02mM propranolol in buffer or 0.1mM in plasma did not produce a change in $P_{50}$.  Propranolol had no effect on hgb-$O_2$ affinity of (a) hemolysates (b) hgb stripped of organic phosphates or (c) hgb solutions with added 2,3-DPG, confirming the requirement of the intact cell for its action (1). 2,3-DPG was not found to be membrane associated in untreated cells.  Indeed, 2,3-DPG/hgb molar ratios were 20% less in sedimented ghosts than in supernatant after hemolysis of untreated or propranolol treated cells.  Propranolol invariably produced 1) a shape change to stomatospherocytes, 2) a reduction in red cell volume ($90\mu^3$ to $70\mu^3$) and $H_2O$ content (67% to 55%).  3)  an increase in cellular hgb (MCHC) (33 to 44 gm/100ml RBC), 4) a 32% increase in 2,3-DPG plus ATP expressed as m moles/ml cell $H_2O$, 5) a 40% decrease in cellular chloride and potassium, 6) a reduction of red cell pH from 7.152 to 7.055 and 7) an increase in $\Delta$pH (pHe-pHi) from 0.223 to 0.465.  The effects of DL or D-propranolol on cell volume , MCHC, $\Delta$pH and hgb-$O_2$ affinity were not prevented by epinephrine. Epinephrine itself had no effect on red cell shape, volume ,$H_2O$ and cation content or $P_{50}$ .

    Other surface-active agents eg. procaine (0.1 to 10mM) produced isovolumic stomatocytosis but had no effect on $P_{50}$.  Hypertonic NaCl added to blood so as to reduce red cell volume to an extent comparable to the volume change with propranolol resulted in 1) increased MCHC (45 gm/100ml) and 2,3-DPG and ATP/cell $H_2O$, 2) decreased intracellular pH (7.095),3) an increased  pH (0.288 and $P_{50}$ (28.00mmHg).  Red cells in hypertonic plasma were crenated discs, not stomatocytes.  Buffered sucrose added so as to shrink red cells to a comparable degree increased MCHC (46 g/100ml) but did not alter intracellular pH (7.170).  The $\Delta$pH was markedly reduced from 0.223 to 0.032 and thereby resulted in a decrease in $P_{50}$ (21.5) when corrected to an extracellular pH of 7.4, but this was largely a factitious shift since intracellular pH (7.170) was similar to control cells (7.152).

    Administration of 20 to 40 mg of propranolol over 4 hours to healthy human volunteers did not result in a change in red cell volume, 2,3-DPG or hgb-$O_2$ affinity when measured within 2 to 24 hours of administration.  This ineffectiveness in vivo was anticipated from the very high concentrations required to achieve effects in blood in vitro (>0.2mM) as opposed to maximal in vivo concentrations after 40 mg propranolol (<0.0005mM).

    Conclusion - 1) Propranolol (0.1 to 0.5mM) induced hypovolumic stomatospherocytes, cellular dehydration and a decrease in intracellular pH.  The decrease in pH is due to a new Gibbs-Donnan equilibrium required by the increased erythrocytic concentration of impenetrant organic anions.  The high concentration of cellular hemoglobin also may have contributed to the change in affinity.  2) The effect of

propranolol on cell hydration and hgb-$O_2$ affinity was not specific in that it was not blocked by epinephrine. 3) ⁻Propranolol administered to normal volunteers did not produce effects on red cell hydration or hgb-$O_2$ affinity.

2.  The affinity of hemoglobin for oxygen may increase significantly in subjects who are hypophosphatemic and alkalotic. We studied the organic phosphate content and oxygen binding by hemoglobin of red cells in subjects undergoing hemodialysis, during which time a decrease in plasma inorganic phosphate and an increase in blood pH may occur.

Red cell 2,3-DPG was not correlated with plasma inorganic phosphorus, whereas red cell ATP was highly correlated with plasma inorganic phosphorus when analyses were made on predialysis samples. Predialysis red cell inorganic phosphorus was highly correlated with plasma inorganic phosphorus, supporting the concept that intraerythrocytic inorganic phosphorus is maintained by a gradient from plasma to cell. Plasma inorganic phosphorus decreased by 45% during the period of hemodialysis; whereas red cell inorganic phosphorus did not change. This finding is compatible with previous studies showing a markedly slower exodus of inorganic phosphorus.

Red cell 2,3-DPG, ATP and oxygen binding by hemoglobin at standard conditions of temperature, pH and $Pco_2$ were not altered after six hours of hemodialysis. Plasma pH and base excess increased during dialysis. The increase in base excess, an estimate of the non-pH dependent effect of $CO_2$ on oxygen binding by hemoglobin, counterbalanced a portion of the effect of elevated pH on hemoglobin-oxygen affinity under in vivo conditions. Hence, only a slight increase in oxygen binding by hemoglobin occurred. Moreover, late dialysis symptoms were not associated with the degree of alkalosis or with the extent of change in hemoglobin's affinity for oxygen. Red cell 2,3-DPG content was lower and hemoglobin's affinity for oxygen was higher in subjects with chronic renal disease than in non-azotemic subjects with similar hemoglobin deficits and increased red cell ATP in chronic renal disease patients did not influence oxygen binding by hemoglobin.

3.  We have found that subjects with hypoproliferative (reticulocytopenic) anemia increased their red cell 2,3-diphosphoglycerate (2,3-DPG) content in relationship to the magnitude of the alkalosis which accompanied their hemoglobin (hb) deficit. Plasma pH increased approximately 0.01 unit for each gram % decrement in blood hb and red cell 2,3-DPG increased 0.64 moles/g hb for each 0.01 unit increment in plasma pH when non-azotemic subjects were studied, confirming previous reports relating blood pH to red cell 2,3-DPG. Subjects with chronic renal disease and severe anemia did not have an increase in red cell 2,3-DPG since their acid load prevented development of respiratory alkalosis despite thrice weekly hemodialysis which maintained their predialysis plasma pH near normal. When all anemic subjects (azotemic and non-azotemic) were considered, red cell 2,3-DPG increased 5.5 moles/g hb for each rise of 0.10 unit of plasma pH.

The $Po_2$ at half-saturation of hb ($P_{50}$) at standard conditions (T=37°C, $P_{CO_2}$ = 40 torr, pH=7.40) increased 1.0 torr for each increment in red cell 2,3-DPG of 3.6 μmoles/g hb; however, $P_{50}$ at in vivo conditions log $P_{50}$ in vivo = log $P_{50}$ std. + 0.48 (7.40 - pH) + .0013 B.E. +.024 (t - 37°C)) did not increase with increasing red cell 2,3-DPG since the plasma alkalosis required to generate increased 2,3-DPG cancelled the effect of the latter on intra-cellular pH. The regression of $P_{50}$ std. on 2,3-DPG for all subjects was $P_{50}$ std. = 0.31 (2,3-DPG + 21.3, whereas the regression of $P_{50}$ i.v. on red cell

2,3-DPG was $P_{50}$ i.v. = .068 (2,3-DPG) + 25.3. Azotemic, anemic subjects administered sodium bicarbonate developed a metabolic alkalosis, (plasma pH as high as 7.54) with accompanying increases in red cell 2,3-DPG (e.g., from 13 to 26 $\mu$moles/g hb) and $P_{50}$ std. (e.g., from 25.0 to 30.5 torr). However, intracellular pH was slightly higher than normal and $P_{50}$ in vivo was slightly less than normal (Table 1).

Conclusions: 1) Blood pH was negatively correlated with hb deficit in non-azotemic subjects with hypoproliferative anemia. 2) Red cell 2,3-DPG was positively correlated with blood pH in anemic subjects and the chain of events: a) reticulocytopenic anemia, b) alkalosis, c)elevated 2,3-DPG could explain, on the average, 75% of the association of $P_{50}$ standard with blood hemoglobin concentration. 3) Azotemic, anemic subjects treated with hemodialysis did not develop alkalosis with anemia and did not have significant elevations of red cell 2,3-DPG and $P_{50}$ standard. If given $NaHCO_3$, chronic alkalosis could be maintained and red cell 2,3-DPG and $P_{50}$ std. increased in relation to the height of the blood pH. 4) $P_{50}$ in vivo was similar in a) healthy, b) anemic, c) hemodialysis and d) hemodialysis subjects using $NaHCO_3$, since the increment in red cell 2,3-DPG and thereby red cell hydrogen ion was counterbalanced by the increment in plasma pH and red cell hydroxyl ion. Indeed, at high blood pH, $P_{50}$ in vivo was slightly less than normal. 5) Changes in hemoglobin's affinity for oxygen which is mediated by 2,3-DPG in reticulocytopenic anemia appears to be in large part a response to alkalosis, and hence does not provide "compensation" for the hemoglobin deficit.

4. The relationships of red cell adenosine triphosphate (ATP) to hemoglobin (hb) concentration and to plasma inorganic phosphate ($P_i$) in azotemic and non-azotemic anemic subjects and subjects with major deviations in plasma $P_i$ but with mild anemia (e.g., alcoholic pancreatitis, liver disease, hyperalimentation) are under study. We have a highly significant increment in red cell ATP with hb deficit anemic subjects (0.37 $\mu$moles ATP/g hb and reduction in blood hb of 1.0 g/100ml; r= -0.89) than in non-azotemic anemic subjects (0.21 $\mu$moles ATP/g hb per reduction in blood hb of 1.0 g/100ml. r=-0.73). The azotemic subjects were all on maintenance hemodialysis and had predialysis plasma $P_i$'s of 0.86 to 2.955 $\mu$moles/ml, whereas the anemic subjects had plasma $P_i$'s of 0.85 to 1.79 $\mu$moles/ml. Red cell ATP was also highly correlated with plasma $P_i$ in azotemic subjects (r=0.75); however. hb and $P_i$ were significantly correlated (r= -0.65): hence, $P_i$ and ATP could be correlated because of a joint association of $P_i$ with both hb and red cell ATP. Using multiple correlation it was found that ATP was not significantly more closely correlated with hb + $P_i$ (r= 0.91), than with hb alone (r= 0.89). Using partial correlation, the association of ATP with hb controlling for an effect of $P_i$ (r = 0.79), was significantly stronger than the association of ATP with $P_i$ controlling for the effect of hb (r = 0.48). Hence about 64% ($r^2$) of the increment in red cell ATP can be explained by hb deficit and 25% by plasma $P_i$ or unknown variables very closely correlated with hb and $P_i$. When studies were made of azotemic subjects, including several made hypophosphatemic with $Al(OH)_3$ gel (plasma $P_i$ 0.5 to 3.0 $\mu$moles/ml), the influence of $P_i$ was heightened. Under these circumstances, red cell ATP was correlated to a similar degree with hb (r= -0.78) and the partial correlation of red cell ATP with hb controlling for the effect of $P_i$ (r= 0.73), and with

$P_i$ controlling for the effect of hb ($r = 0.70$), were also similar.

Increased red cell ATP was not explained by the percent of reticulo-cytes since in previous studies in patients with hemolytic anemia, we observed that red cell ATP increased by 0.09 μmoles/g hb for each 1% increment in reticulocytes. Anemic subjects in this study had less than 5.0% reticulocytes.

Red cell, 2,3-diphosphoglycerate (2,3-DPG) was not correlated with plasma $P_i$ between 0.5 and 3.0 μmoles/ ml in severely anemic subjects with and without azotemia. In subjects with pancreatitis, alcoholic liver disease and hyperalimentation with plasma $P_i$ below 0.33 μmoles/ml, red cell 2,3-DPG was below normal despite mild anemia and in some cases, plasma alkalosis. The latter two variables are usually associated with elevated red cell 2,3-DPG. Red cell ATP was significantly reduced in proportion to the extent of reduction in plasma $P_i$.

Red cell ATP did not contribute to oxygen binding to hb in intact cells. Regression of $P_{50}$ std. (i.e., the $Po_2$ at which hb is 50% saturated with oxygen at 37°C, pH = 7.40, $PCO_2 = 40$ torr) on red cell ATP was nil ($r = 0.02$), whereas that of $P_{50}$ std. on red cell 2,3-DPG was highly significant ($r = 0.81$). The correlation of $P_{50}$ with 2,3-DPG + ATP was significantly less than that of 2,3-DPG alone. Several subjects with chronic renal disease on hemodialysis with normal red cell 2,3-DPG content had a red cell ATP content greater than 4.0 μmoles/g hb above that of healthy subjects without an increase in $P_{50}$ std. One non-azotemic, anemic subject with leukemia had a severe hb deficit (3.5g/100ml) normal red cell 2,3-DPG, a marked increase in red cell ATP (3.5 μmoles/g hb above normal) without an increase in $P_{50}$ std. on repeated examination.

Red cell magnesium ($Mg^{2+}$) and red cell pH were measured in ten anemic subjects (nine of whom were receiving dialytic therapy) with high red cell ATP ($> 6.5$ μmoles/g hb and 2,3-DPG within the normal range. Red cell $Mg^{2+}$ was significantly greater than in healthy subjects and $Mg^{2+}$/ATP ratios were above 1.3. High ATP red cells did not have a reduction in pH at an extracellular pH of 7.40 (i.e., $pH_e$ - $pH_i$ gradient was not greater than 0.23). The gradient was increased in red cells with elevated 2,3-DPG. A regression of $P_{50}$ on 2,3-DPG was made by incubating blood in inosine (10mM), pyruvate (5mM) and phosphate (3mM). $P_{50}$ and 2,3-DPG measurements were made simultaneously at various times over nine hours. 2,3-DPG rose to as high as two times normal at nine hours. Incubation of blood with adenine (10mM), pyruvate (5mM) and $P_i$ (30mM) resulted in elevation in red cell ATP at nine hours to as high as three times normal whereas 2,3-DPG increased only 1.3 times normal. $P_{50}$ after adenine, pyruvate and $P_i$ was slightly greater than expected from the increase in 2,3-DPG. $Mg^{2+}$/ATP ratios were 0.75 to 0.95.

Hence, 1) Red cell ATP is increased in subjects with hypoproliferative anemia in relationship to hb deficit. The increase cannot be explained by the presence of increased proportions of reticulocytes, although average cell age may be a factor. 2) Increased plasma $P_i$ influences only slightly the red cell ATP in azotemic subjects; however, this factor or a closely correlated one may explain the greater increment in red cell ATP with anemia and if severe enough, will reduce red cell ATP, and when reduced becomes an important factor in the determination of red cell ATP level. 3) ATP

did not influence $P_{50}$ of intact red cells, perhaps because of the known inability of $Mg^{++}$-ATP to bind to specific sites on hemoglobin. 4) 2,3-DPG was not correlated with plasma $P_i$, unless $P_i$ was below 0.33 µmoles/ ml.

5 a) Our previous reports have dealt with observations related to studies of oxygen binding by hemoglobin. These studies are being continued and extended. In particular the role of adenosine triphosphate in vivo in the regulation of oxygen binding to hemoglobin is being explored extensively in order to answer this important question.

b) The role of dynamic changes in hemoglobin oxygen affinity in the maintenance of oxygen consumption in subjects with acute myocardial infarction is being studied. The causal relationship of blood pH and the time-averaged deoxy to oxyhemoglobin ratio to red cell 2,3-DPG content is being examined as an intermediary mechanism which determines the decrease in hemoglobin-oxygen affinity which occurs with decreasing arterial oxygen flow rate following myocardial infarction. The quantitative role of changes in affinity to increased proportional extraction of oxygen with decreasing arterial oxygen flow rate is being assessed.

c) The frequency of occurrence and effect of respiratory alkalosis in subjects with trauma are being examined to determine if acute increases in affinity leads to a reduction in oxygen consumption and/or whether increases in blood flow compensate for increased affinity.

d) The role of radio opaque dyes in the apparent increase of hemoglobin-oxygen affinity has been examined. This has been shown to be a factitious effect which is inconsequential in vivo. The importance of direct measurement of red cell pH when studying the effect of agents which disturb the normal electrical potential of the red cell membrane has not been previously appreciated.

6. Studies of the role of red cell aging in flow in capillary tubes have been performed. Erythrocyte flow in capillaries may depend significantly on membrane elasticity, cell surface-capillary wall resistance and the character of the thin lubricating film separating membrane and capillary surface. The relatively rigid senescent RBC would be expected to have abnormal flow characteristics. We examined membrane elasticity in terms of unidimensional extensibility of membrane as a function of time and force, determined the erythrocyte flow velocity ($V_{RBC}$) and derived values of membrane capillary wall resistance (C) and lubricating film thickness ($\delta$ ) in order to compare capillary flow of senescent to normal RBC. Membrane extensibility was measured by aspiration of a portion of RBC into a 0.5 µm micropipette at pressures 0-10mmHg and determination of membrane elongation with time intervals 10-300". $V_{RBC}$ was recorded directly for RBC flowing in a 2.8 µm glass micro capillary; suspended in a 2mM Tris-NaCl isotonic buffer containing 0.25gm% albumin and γglobulin concentrations 0-10gm% at pressure differentials 0-3mm $H_2O$. The extension of senescent cells increased more rapidly than control cells and whereas the extension ratio$^2$($\gamma x^2$) for control membrane was linear with force, in senescent cells initial resistance to extension, then rapid ↑ of extensibility occurred and at times >120" plastic flow of membrane was observed.

RBC for control was 6-15 $\mu m/sec$, 1/2 that of cell free axially flowing fluid, and $V_{RBC}$ for senescent cells was identical: 5-17 $\mu m/sec$. Addition of globulin did not affect $V_{RBC}$ or C which was $10^4$ dyn $sec/cm^3$. $\delta \equiv 10^{-2}m$ for both control and senescent RBC. These data indicate different elastic properties of senescent cells and predict retardation of flow in critical channels where time dependent extensibility is important. Normal and senescent cells have similar flow characteristics in capillaries >2.8 $\mu m$ and are not affected by protein concentration.

We have also examined the mechanism of poikilocyte formation in thalassemic and sickle red cells.

Erythrocytes undergo both rapid and protracted deformation during flow. Elasticity, essential for protracted deformation, depends on membrane molecular interactions and may be changed by alteration of membrane and stress duration. These studies compared immediate and time dependent membrane extensibility as a measure of elasticity in normal, sickle and thalassemic cells and examined their flow velocity in in vitro glass microcapillaries, to determine whether significant change of elasticity correlates with duration of stress, permanent deformation (poikilocyte) and altered flow. Elasticity was measured as $\gamma$ length of membrane extension, aspirated into 0.5 $\mu$micropipette at pressures up to 100 $mmH_2O$, at times 30", 5-30', and residual length after force removal. Flow velocity was determined for cells in plasma at T=23°C. 30" (immediate deformation) $\gamma$ values for 50 cells: control, 2.2; SS(ISC), 1.1; SS (reversible), 2.1; Th, 1.8. Na cyanate improved $\gamma$ in deoxy RSC. Permanent deformation occurred in ISC and Th. V for control cells was 3.6+0.2mm/sec/$mmH_2O$ in a 2.8 $\mu$microcapillary. V for oxy SS(RSC) and Th was normal; at $pO_2$=20-25mmHg, SS(RSC)=3.2+0.7; 32% did not enter the microcapillary. V=3.2+0.5 for 42% of ISC which enter. Reduced immediate and time dependent extension of pathologic cells confirms prediction of abnormal elasticity in abnormal membranes. Permanent membrane distortion (residual $\gamma$) and fragmentation at low forces at 5-30' explain how in vivo poikolocyte formation and fragmentation can occur even at low stresses of the microcirculation when stress is protracted.

7.  The relationships of hemoglobin concentration and blood pH to red cell 2,3-diphosphoglycerate and oxygen binding by hemoglobin have been studied in healthy subjects and subjects with hypoproliferative anemia with or without severe chronic renal disease.  Red cell 2,3-DPG was inversely correlated with hemoglobin deficit and directly and equally strongly associated with blood pH in anemic subjects without chronic renal disease.  In subjects with chronic renal disease receiving regular hemodialysis, predialysis pH was not increased despite severe anemia, and red cell 2,3-DPG was not significantly elevated, except in subjects who had a sustained alkalosis due to the use of sodium bicarbonate.

In hypoproliferative anemia, the increment in pH was associated with the decrease in hemoglobin concentration such that 80% of the increase in $P_{50}$ measured at standard conditions which occurred with anemia was explicable by the relationship of (a) pH with hemoglobin concentration, (b) red cell 2,3-DPG with pH, and (c) $P_{50}$ std with red cell 2,3-DPG.  However, $P_{50}$ at the pH and base excess present in vivo was similar in all anemic subjects whether an increase in red cell 2,3-DPG occurred or not.  Blood alkalosis and the accumulation of 2,3-DPG cancelled each other's effect on oxygen binding by hemoglobin.  Hence, increased red cell 2,3-DPG and $P_{50}$ compensated for the alkalosis of hypoproliferative anemia, not for the deficit in hemoglobin concentration.

8.  We have shown that propranolol reduces oxygen binding by hemoglobin in intact red cells by increasing the selective permeability of the red cell membrane resulting in an exodus of potassium, chloride, and water.  The latter effects result in a new distribution of hydrogen ion between cell and plasma, and thereby a reduction in red cell pH.  The reduction in pH can fully explain the change in hemoglobin's affinity for oxygen based on the Bohr effect.  Either D- or DL-propranolol can produce the change in red cell pH and oxygen binding by hemoglobin.  The drug action on permeability is not prevented by epinephrine, although it is by chlorbutanol.  Hence, the membrane action of propranolol does not appear to be related to its activity as a beta-adrenergic receptor blocking agent.

Propranolol produced a marked alteration in red cell shape as well as in hydration (hypovolumic stomatocytes).  The two effects were separable since dehydration of the cell by the addition of sucrose to plasma did not result in stomatocytes and chlorbutanol blocked the enhancement of permeability of the red cell membrane by propranolol without preventing the shape change (isovolumic stomatocytes).  This suggests that propranolol may have two separate sites of membrane interaction.

Propranolol (10 to 360 mg) administered to human subjects did not affect hemoglobin-oxygen affinity.  This is explained by the fact that the concentration in blood after such doses is nearly 4,000 to 100-fold lower than that required to achieve changes in blood in vitro.

9. The interrelationships of arterial oxygen flow rate index, oxygen binding by hemoglobin, and oxygen consumption have been examined in patients with acute myocardial infarction. Proportional extraction of oxygen increased in close association with decreasing oxygen flow rate, and hence, whole body oxygen consumption was constant over nearly a three-fold variation in arterial oxygen flow rate. A reduction in hemoglobin-oxygen affinity at in vivo conditions of pH, $Pco_2$ and temperature also occurred in proportion to the reduction in arterial oxygen flow rate. Therefore, the increased proportional removal of oxygen from arterial blood at low oxygen flow rates, required to maintain oxygen consumption, may have been facilitated by the reduced affinity of hemoglobin for oxygen at in vivo conditions. However, the decrease in affinity did not appear to explain more than 30-40% of the increased extraction.

Respiratory alkalosis was a frequent occurrence in these patients and 2,3-diphosphoglycerate was positively associated with blood pH as well as with the time-averaged proportion of deoxyhemoglobin in arterial and venous blood.

Hemoglobin-oxygen affinity measured at standard conditions and the mixed venous oxygen saturation were equally good indicators of reduced arterial oxygen flow rate in patients without shock. However, $S\bar{v}o_2$ is more easily measured and is a more useful indicator of reduced oxygen flow rate, since its relationship to oxygen flow appears to be independent of affinity changes and time.

10. The inconvenience of measurement of red cell pH, has led to the practice of establishing the relation of oxygen saturation of hemoglobin to the partial pressure of oxygen in blood based on extracellular pH. This method relies on the precise dependence of intraerythrocytic pH on extracellular pH. Studies of the effects of certain plasma additives on the binding of oxygen to hemoglobin have neglected to consider the possibility that the normal negative potential difference between the interior and exterior of the red cell may be disturbed by the agent under study. The change in potential leads to a concomitant loss in the usual relationship between extracellular and intracellular ionic species and thereby pH.

In a recent study of the effects of radiographic contrast media on oxygen binding to hemoglobin, an increased affinity of hemoglobin for oxygen was observed based on corrections using extracellular pH. However, it would be expected that poorly penetrating compounds would reduce the pH gradient between plasma and cells, by causing an acidification of plasma due to a net movement of hydroxyl ion into the red cell without a concomitant change in red cell pH because of the high buffering capacity of the red cell interior. Hence, a portion of the apparent affinity change would be spurious, since intracellular pH governs oxygen binding to hemoglobin.

The following studies demonstrate how erroneous inferences may be drawn from reliance on extracellular pH for determination of the oxygen-hemoglobin dissociation curve in situations where the ion distribution across the red cell membrane is disturbed.

Construction of oxygen-hemoglobin dissociation curves based on extracellular pH and using blood tonometered with 5% $CO_2$ was shown misleading in the presence of poorly penetrating non-ionic molecules like sucrose or poorly penetrating anionic compounds like radiographic contrast materials. False conclusions regarding the position of the oxygen-hemoglobin dissociation curve result because of the disturbance of the normal pH gradient between plasma and red cell induced by such chemicals.

11. The development and use of radiographic contrast media have been major contributions to clinical medicine. Although considerable study has been given to the adverse effects of these agents, some reactions remain unexplained. Most research on the adverse effects of water-soluble contrast materials has been directed to the effects of the rapid injection of a hyper-viscous and hypertonic bolus on rheology of blood, although a "tendency to acidosis" has been noted previously.

The effect of water-soluble radiographic contrast material on pH when added to blood in clinical dosages in vitro or when used in vivo for diagnostic purposes, was examined. Contrast material caused a reduction of blood pH. The mechanism of this occurrence was found to be the balancing of the negative charge of intracellular organic anions by the extracellular anionic contrast material molecules. The normal negative potential of about 10 millivolts across the red cell membrane was reduced, nullified or reversed depending on the concentration of contrast material, added to blood. As the inside of the cell became more positive with respect to the outside, protons were, in effect, repelled into plasma, although the apparent exodus of protons occurs by the generation and outward diffusion of carbon dioxide. Since the acidemia is dependent on rehydration of carbon dioxide in plasma, a reaction measured in seconds, the site of injection and transit time of dye will contribute to the pH of the plasma during passage through a regional capillary bed.

We speculate that an alteration in membrane potential and/or the acute acidemia may contribute to the adverse effects of contrast material, particularly on tissues dependent on membrane electrical rhythmicity such as the myocardium.

12. The oxygen-hemoglobin equilibrium is of clinical importance since it may provide evidence for a structurally altered hemoglobin with heightened or lessened affinity for oxygen. The presence of a structurally altered hemoglobin with abnormal oxygen binding characteristics is made evident by examining the oxygen-hemoglobin dissociation curve at standard conditions of temperature, pH and $Pco_2$. The position of the curve is represented by the $P_{50}$ standard ($P_{50}$ std), that is the $Po_2$ at which hemoglobin is half-saturated with oxygen at standard conditions. Standardization eliminates the effects of variations in temperature, pH and the pH-independent effect of $Pco_2$. Deviations in the $P_{50}$ std, therefore, imply either an altered content of red cell 2,3-DPG or a hemoglobin with oxygen binding characteristics different from hemoglobin A.

In addition, the affinity of hemoglobin for oxygen is of clinical importance because it may decrease in response to hypoxia, anemia or reduced blood flow and acts, thereby, to maintain venous (i.e. tissue) $Po_2$ as

oxygen extraction increases. In situations concerning oxygen-hemoglobin content or flow, the effects of the four major determinants of the equilibrium, that is red cell 2,3-DPG, pH, temperature, and $Pco_2$ must be considered. This has been called the oxygen-hemoglobin dissociation curve at in vivo conditions, and is presented by the $P_{50}$ in vivo ($P_{50}$ i.v.).

An assessment of the affinity of hemoglobin for oxygen is considered inaccessible to the practicing physician and hematologist since it requires tonometry and mixing techniques often available only in research laboratories. In the following studies, we examined the usefulness of a single venous blood sample as an indicator of the position of the oxygen-hemoglobin dissociation curve. A single venous blood sample, analyzed for pH, $Po_2$, and $So_2$ by a clinical chemistry laboratory could be used by any physician to assess the presence of an alteration in the oxygen-hemoglobin equilibrium.

We have validated this technique correlating the $P_{50}$ std or $P_{50}$ in vivo derived from an          -hemoglobin dissociation curve with that from a single measurement of pH, $Po_2$, $Pco_2$ and $So_2$ in venous blood. Studies of subjects with alkalosis or acidosis and with high and low affinity hemoglobins were made to verify this technique. Equations were developed to allow the calcula-tion of $P_{50}$ from a single $Po_2$ and $So_2$.

## 13. Atrial tachypacing and oxygen-hemoglobin affinity.

The first study deals with atrial tachypacing and oxygen-hemoglobin affinity in patients with chest pain syndromes, most often with angina pectoris. It was conceived originally in response to research reported by Shappell and coworkers and referred to earlier in this application. Their studies showed that 6 patients with coronary disease who underwent atrial tachypacing, only the 5 who developed chest pain showed an increase during pacing in $P_{50}$ standard from arterial to coronary sinus blood. The increase was independent of DPG, ATP, or red cell pH--the known determinants of $P_{50}$ standard--and suggested the possibility that a substance was released during ischemia which produced the decrease in oxygen hemoglobin affinity across the coronary bed. Because of the potential significance of this finding (in particular if right shifted curves are in fact advantageous in ischemic states), and because investigations in our laboraotry indicated that $P_{50}$ standard correlated very strongly with DPG alone, we were interested in further exploring this question in patients undergoing atrial tachypacing studies for diagnostic purposes in our cardiac catheterization laboratory, where routine coronary sinus pacing makes the requisite blood sampling possible and convenient. Thus far, 11 patients, 9 with coronary artery disease and pacing induced angina, and 2 with chest pain and normal coronary arteries, have been studied in control, paced, and recovery states. An initially surprising (and to our knowledge new) observation, is that virtually each patient developes alkalosis during pacing, and this pH change is on a respiratory basis ($pCO_2$ falls appropriately). A rise in arterial $pO_2$ from the rest to the paced state occurred in many patients and is consistent with this increase in respiration. As a consequence of the pH change, $P_{50}$ at in vivo pH (calculated from $P_{50}$ standard) is decreased during atrial tachypacing. Unlike the prior investigations, we have not detected during the paced state any increase in $P_{50}$ standard from artery to coronary sinus. Without enumerating the several ways in which apparently differing results

can be reconciled, the results of this study appear to be of interest in at least two respects. First, as indicated in the original proposal, it is conceivable that when flow is fixed (as it well may be in the regionally involved areas in coronary heart disease), and when oxygen extraction is maximum, a leftward shift in the in vivo oxygen hemoglobin dissociation curve (such as that induced by alkalosis) may be detrimental to oxygen transport. Making several assumptions (regional flow is fixed, venous $pO_2$ is proportionate to tissue $pO_2$, and for both there is a critical ischemic level, and capillary $pO_2$ is rate limiting for oxygen transport to ischemic tissue), we calculated that the patient with the largest decrease in $P_{50}$ in vivo (arterial, coronary sinus, and averaged) during pacing might have decreased his oxygen extraction and therefore his oxygen transport by 10% as a result of the alkalosis. Using this reasoning, it is conceivable that pH through its effect on oxygen delivery (just as rate pressure product has its effect on oxygen demand) is important in determining threshold for angina. Second, it is also conceivable that in spontaneous angina there may be pH (alkalosis) antecedents, just as there are blood pressure and heart rate antecedents, and that these may effect threshold for pain by impairing oxygen transport. Pilot studies are planned monitoring depth and rate of respiration in hospitalized patients with ischemic chest pain to explore this latter possibility.

14 Chronic cardiac decompensation and oxygen-hemoglobin affinity

A clinical study is underway exploring relationships between 2,3-DPG, pH, oxygen hemoglobin affinity, and oxygen transport in patients with varying degrees of chronic cardiac decompensation.

Earlier studies have established that 2,3-DPG and $P_{50}$ standard increase in patients with cardiac decompensation. Woodson and coworkers noted that the increase in $P_{50}$ standard was greater in patients with more severe disease, and that it correlated best with mixed venous oxygen saturation. pH was not measured in this study, and therefore, its role in producing affinity changes could not be assessed, and $P_{50}$ in vivo could not be calculated from $P_{50}$ standard. Rosenthal and coworkers noted a significant inverse relationship between oxygen flow index and 2,3-DPG, but as in the previous study, the role of pH was not assessed.

The study which we have planned, involving patients with chronic heart disease undergoing diagnostic cardiac catheterization at Strong Memorial Hospital, is designed to be comprehensive in a fashion analogous to that previously reported involving patients with acute cardiac decompensation following myocardial infarction (including measurement of cardiac index, hemoglobin, arterial and mixed venous blood gases, and arterial and mixed venous red cell ATP, 2,3-DPG and pH). In particular, the questions to be explored are the following:

(1) What are the interrelations between arterial oxygen flow rate (cardiac index times arterial oxygen content), oxygen hemoglobin affinity, and oxygen consumption?

(2) If oxygen extraction increases as cardiac index falls, to what extent can this extraction be accounted for in decreases in in vivo oxygen hemoglobin affinity?

(3) What is responsible for the increase in 2,3-DPG which accompanies a fall in arterial oxygen flow index (cardiac index and arterial oxygen content)? Does the increase in 2,3-DPG correlate with blood pH (arterial or averaged arterial and mixed venous)? Does it correlate with time averaged percent deoxyhemoglobin in arterial and mixed venous blood? By implication, is pH or percent deoxyhemoglobin or a combination of both the driving force for the increase in 2,3-DPG? Are there other factors involved.

(4) What is in vivo P50? How do pH and DPG changes interact in determining in vivo $P_{50}$? Do changes in $P_{50}$ in vivo correlate with arterial oxygen flow index and/or arterial oxygen content?

(5) How does oxygen transport compare in equivalent degrees of acute and chronic cardiac decompensation?

Fifteen patients have been studied thus far, but the results are in too preliminary a state of analysis for comment at this time.

15. Cardiac and skeletal muscle models for studies of $O_2$ metabolism

a. Myocardial model

The coronary sinus could be cannulated and the cannula sutured in place. In a significant number of instances, the left main coronary artery could be dissected, and a catheter introduced from the carotid artery could be sutured in the dissected coronary vessel. Atrial pacing, epicardial ST mapping, and measurement of pressures were performed as planned.

Two major problems, however, were encountered. First, catheters available from our animal research and cardiac catheterization laboratories offered too great a resistance to flow and prevented adequate perfusion of the coronary circulation; cardiac arrest followed soon after cannulation. Subsequently, a stainless steel modified Gregg cannula has been made to order according to specifications provided by Dr. Peter Maroko, but this cannula has not been used as yet. Second, no satisfactory way was found to produce an in vivo change in either DPG or $P_{50}$. Inosine, phosphate, and pyruvate, in .1 Molar concentrations each produced no change in DPG in the dog, presumably because of the absence in this species of the enzyme erythrocyte purine nucleoside phosphorylase (required for the transformation of inosine into ribose phosphate and then into 2,3-DPG). Sodium bicarbonate in daily intravenous doses, did increase 2,3-DPG levels, but correction of the alkalosis to a stable pH in the normal range with ammonium chloride prior to the acute experiment proved difficult. pH alone could not be used to induce an in vivo affinity change because of the varied effects of pH itself. For these two reasons--initially, lack of a suitable perfusion cannula and later, the absence of a suitable means of inducing in vivo changes in $P_{50}$ and, in addition, because practical considerations suggested working with one model at a time, the major portion of our effort in the laboratory has been devoted to developing the gracilis muscle model, described below, where progress has been more rapid. When the gracilis studies are well underway, we plan to continue to work with the coronary model.

**b.** Gracilis model

### (i) The experimental design

The design is essentially as described in the contract proposal of 1974-1975. Dissection and cannulation are now performed with facility. Blood flow is arbitrarily fixed at 5 cc per minute, a flow which is adequate for tissue oxygenation at rest and at which oxygen transport is limited during contraction. Sodium bicarbonate rather than saline is used for volume expansion during the exsanguination because of the tendency for metabolic acidosis to develop. Red cells resuspended in plasma have proven superior as a perfusate to those suspended in Krebs-Ringer solution. Oxygenation of the blood and $pCO_2$ control are achieved by bubbling a gas mixture of 4% carbon dioxide and 96% air through two Dow oxygenators in series (Dow - Beaker Gas Permeator - D/HFG-1 silicone hollow fibers). (Pilot studies in our laboratory showed that one oxygenator did not provide adequate gas exchange to maintain equilibrium with perfusate flowing through the system at 5 cc per minute. With two in series, equilibrium could be reached within 30-45 minutes and maintained after flow was initiated). To further improve pH control, (beyond the substantial control achieved with $pCO_2$ regulation), a Radiometer titrator (titrator - type TTT1C and auto-burette type ABU-1B), is now being incorporated into the system. Perfusate is warmed to 32° by passing perfusion lines through a constant temperature bath and this temperature matches closely that of the muscle, which is maintained by radiant heat and measured with needle probes in the muscle. (Alterations in design are planned to bring the temperature of the experiment to 37°). Resting oxygen consumption of the muscle, perfused and maintained in this fashion is 1.2 μl/min-gm (Mean, S.E. .15, n=11). This value obtained at 32° (if a Q10 of 2 is taken as a reasonable estimate), is comparable to that described at 37° by other investigators. The muscle autoregulates appropriately to changes in flow, to occlusion, and to contraction (examples of this auto-regulation are shown in the "controls" section).

With regard to contraction, the 20 minutes of stimulation at 2 twitches per second, which was originally proposed, has proven to be excessive (the muscle cannot maintain tension, and recovery from contraction is prolonged). The experimental protocol has now been modified to 45 seconds of contraction at 1 twitch per second, a format where twitch tension is maintained, metabolic recovery is achieved within 30 minutes, and recovery of vascular resistance occurs in a significantly shorter time. Oxygen consumption during contraction is 5-10 times that at rest.

### (ii) Control runs

Control runs using blood untreated except for initial titration to pH 7.4, have demonstrated that in a given muscle, each of three successive sets of 45 seconds contraction and 30 minutes recovery is quite similar. They are similar with regard to (1) autoregulation to occlusion prior to contraction (magnitude of the response to a standard 30 seconds occlusion). (2) resting oxygen consumption and resting metabolism, (3) tension generated, fall in vascular resistance, and increase in the metabolism during contraction, and (4) the time course of recovery of vascular resistance after contraction. The following is the data from one such control run:

The muscle was prepared in the manner just described and the aforementioned protocol was followed for four successive sets of contraction. Autoregulation in response to a 30 second occlusion was tested prior to each set, a five minute period of recovery from the occlusion followed, then 45 minutes of one contraction per second, followed by a 30 minute recovery period, at which time autoregulation to occlusion was tested again and a cycle repeated. The muscle temperature was 32°, flow fixed at 4.8 cc/min, and hemoglobin 12 grams/100 ml.

### Metabolism

Table 1 shows the metabolic data for the experiment. "Contraction" samples were obtained during the last 15 seconds of contraction and the first 30 seconds of recovery. The values for the arterial sample during contraction are assumed to be identical to the arterial control (i.e., $A_1 = A_2$, $A_4 = A_5$ $A_7 = A_8$; this has in fact, been documented in pilot studies). Recovery samples from one set of contraction are used as controls for the next (i.e., $A_3$, $V_3$ is also taken as $A_4$, $V_4$; the latter values, not actually measured, are placed in parentheses). Metabolic data were obtained for the fourth set in which inadequate volume of perfusate was available for the recovery period.

The remarkable similarity between control and recovery samples (i.e., $A_1$, $V_1$ and $A_3$, $V_3$, $A_4$, $V_4$ and $A_6$, $V_6$) and between successive "contraction" samples ($V_2$, $V_5$, $V_8$) should be noted.

Mechanical performance and autoregulation--The subsequent four pages-- one page for each of the four sets of contraction--consist of photocopies of the original Brush recordings (Clevite-Brush--Mark 260) of perfusion pressure and muscle tension during autoregulation to occlusion, contraction, and the early phase of recovery (paper speed 1mm/second). The response to autoregulation (at the top of the page) occurs prior to contraction (at the bottom), and the tracings are not continuous. This autoregulation to occlusion is quantified in terms of initial perfusion pressure (proportional to initial resistance, since flow is constant, and labelled $R_i$), minimal perfusion pressure upon reinstitution of flow ($R_a$), $\Delta R = R_i - R_a$, and $\Delta R/R_i$. The scale for recording tension is determined at the beginning of the experiment; at present we are not calibrating tension against a known standard, and while successive sets of contractions can be compared quantitatively with one another, no absolute values can be assigned to the magnitude of the tension generated. The first derivative of tension generated with regard to time, $dT/dt$, is also obtained, through an electronic differentiating circuit. (The sudden change in $dT/dt$ in the first set of contractions is due to adjustment of the scale while muscle is being stimulated). Tension generated during contraction is expressed as $T_i$ (tension generated in the second twitch) and $T_f$ (tension of the final twitch). The resistance change with the autoregulation to contraction is expressed as $R_i$ (initial perfusion pressure), $R_f$ (perfusion pressure at the end of contraction), $\Delta R = R_i - R_f$, and $\Delta R/R_i$. Again, the reproducibility in successive sets of occlusions and contractions should be noted.

Table 1

| | CONTROL | | CONTRACTION | | RECOVERY | |
|---|---|---|---|---|---|---|
| | $A_1$ | $V_1$ | $A_2$ | $V_2$ | $A_3$ | $V_3$ |

Contraction Set #1

| | | | | | | |
|---|---|---|---|---|---|---|
| pH | 7.42 | 7.39 | -- | 7.38 | 7.41 | 7.40 |
| $pO_2$ mmHg | 175 | 52 | -- | 21.5 | 175 | 41.5 |
| $pCO_2$ mmHg | 49.5 | 47 | -- | 52 | 45 | 48 |
| % sat. | 99 | 85 | -- | 24 | 99 | 83.5 |
| Q ml/min | 4.8 | -- | -- | 4.8 | -- | 4.8 |
| AV $O_2$ diff. | 14% | -- | -- | 75% | -- | 15.5% |
| $\dot{V}_{O_2}$ $\mu$l/min/gm | 1.8 | -- | -- | 9.6 | -- | 2.0 |

| | $A_4$ | $V_4$ | $A_5$ | $V_5$ | $A_6$ | $V_6$ |
|---|---|---|---|---|---|---|

Contraction Set #2

| | | | | | | |
|---|---|---|---|---|---|---|
| pH | (7.41) | (7.40) | -- | 7.40 | 7.42 | 7.41 |
| $pO_2$ mmHg | (175) | (41.5) | -- | 21 | 175 | 46 |
| $pCO_2$ mmHg | (45) | (48) | -- | 51 | 48 | 48 |
| % sat. | (99) | (83.5) | -- | 25 | 100 | 84 |
| Q ml/min | (--) | (4.8) | -- | 4.8 | -- | -- |
| AV $O_2$ diff. | (--) | (15.5%) | -- | 74% | -- | 16% |
| $\dot{V}_{O_2}$ $\mu$l/min/gm | (--) | (2.0) | -- | 9.5 | -- | 21 |

| | CONTROL | | CONTRACTION | | RECOVERY | |
|---|---|---|---|---|---|---|
| | $A_7$ | $V_7$ | $A_8$ | $V_8$ | $A_9$ | $V_9$ |

Contraction Set #3

| | | | | | | |
|---|---|---|---|---|---|---|
| pH | (7.42) | (7.41) | -- | 7.42 | 7.38 | 7.40 |
| $pO_2$ mmHg | (175) | (46) | -- | 22 | 175 | 42 |
| $pCO_2$ mmHg | (48) | (48) | - | 48 | 50 | 45 |
| % sat. | (100) | (84) | -- | 26 | 99 | 81 |
| Q ml/min | (--) | (--) | -- | -- | -- | -- |
| AV $O_2$ diff. | (--) | (16%) | -- | 74% | -- | 18% |
| $V_{O_2}$ $\mu$l/min/gm | (--) | (21) | -- | 9.5 | -- | 2.3 |

Contraction Set #1

Autoregulation to 30 sec occlusion

$R_i = 110 \quad \Delta R = 50$
$R_a = 60 \quad \Delta R/R_i = 50/110 = .45$

Perfusion pressure

100
50
0

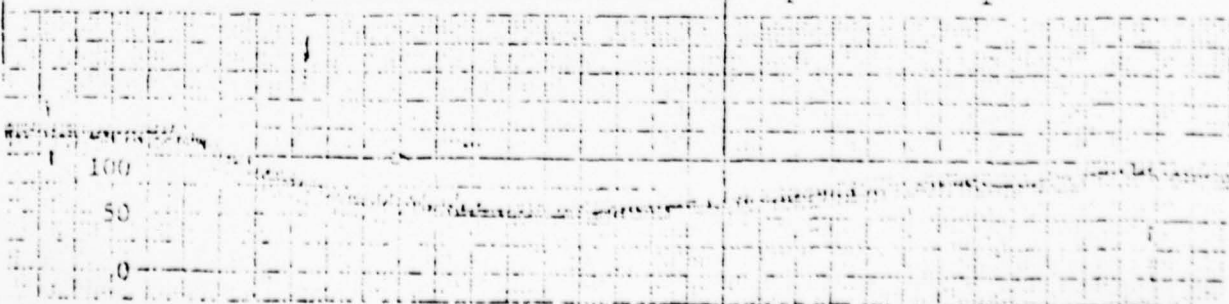Contraction - 45 sec    1 twitch/sec
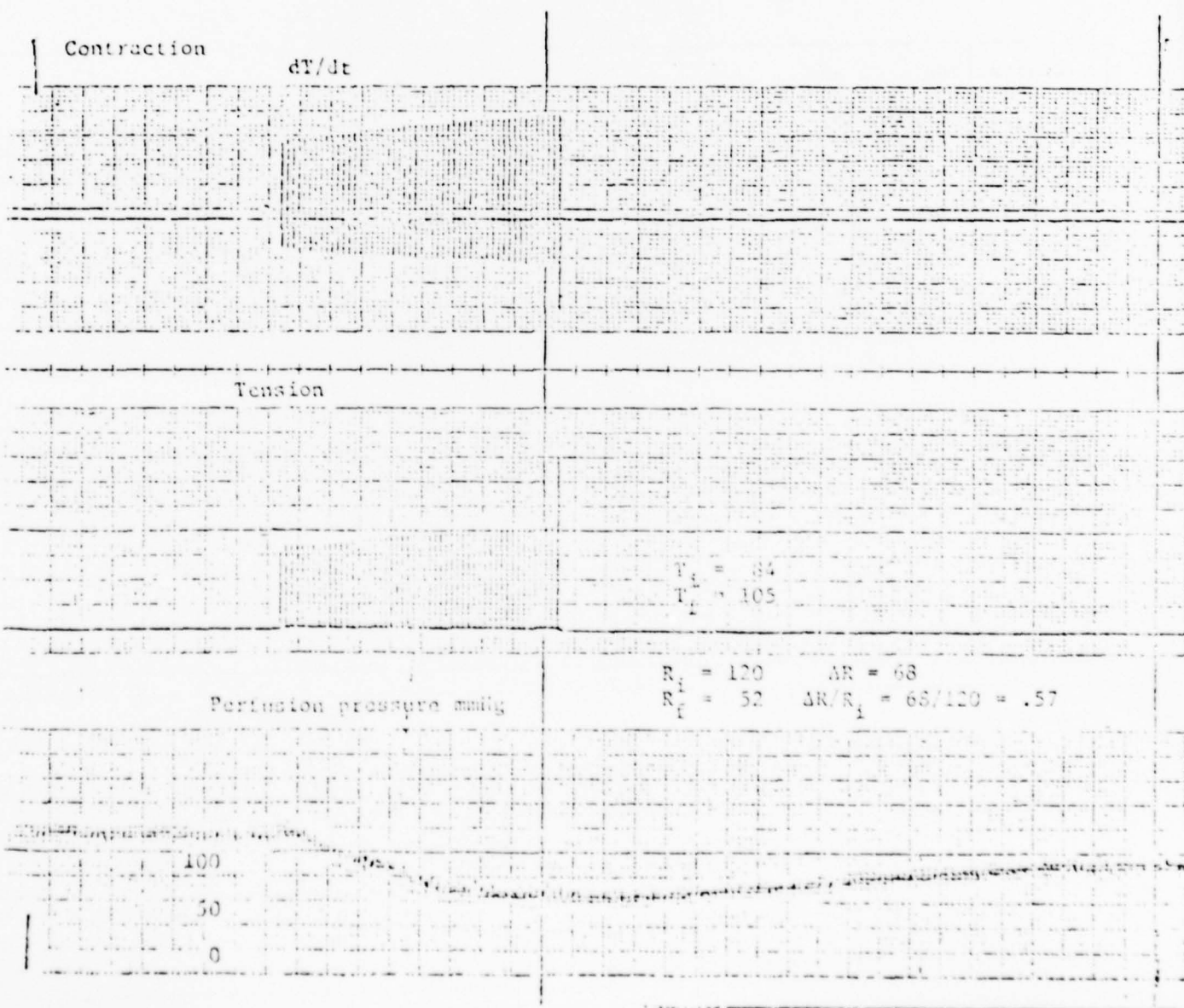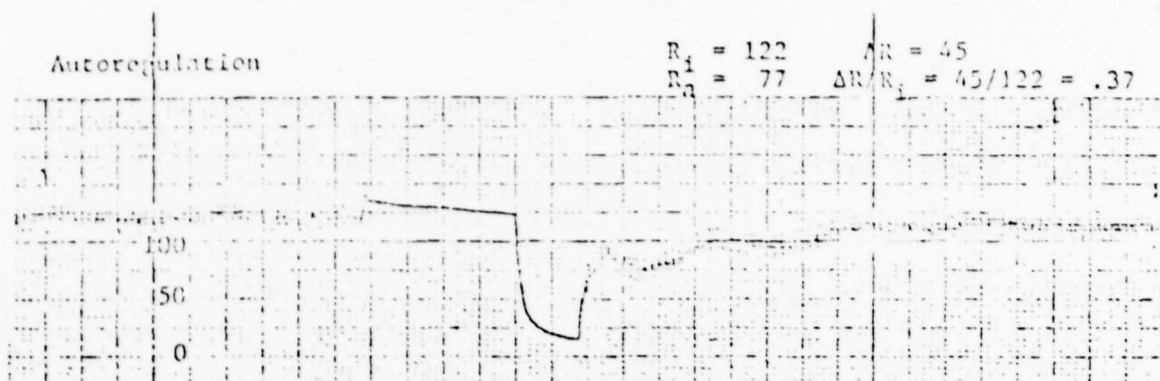
dT/dt

artifact (scale adjustment)

Tension

$T_i = 88$
$T_f = 100$

Perfusion pressure  mmHg

$R_i = 115 \quad \Delta R = 60$
$R_f = 55 \quad \Delta R/R_i = 60/115 = .52$

100
50
0

Contraction Set #2

Autoregulation

$R_1 = 122$     $\Delta R = 45$
$R_2 = 77$     $\Delta R/R_1 = 45/122 = .37$

100

50

0

Contraction     dT/dt

Tension

$T_1 = .84$
$T_2 = 105$

Perfusion pressure mmHg

$R_1 = 120$     $\Delta R = 68$
$R_2 = 52$     $\Delta R/R_1 = 68/120 = .57$

100

50

0

Contraction Set #3

Autoregulation to occlusion

$R_i = 115$    $\Delta R = 62$

$R_a = 57$    $\Delta R/R_i = 62/115 = .53$

100
50
0

Contraction - 45 sec

dT/dt

Tension

$T_i = 92$

$T_f = 115$

$R_i = 117$    $\Delta R = 62$

$R_f = 85$   $\Delta R/R_i = 62/117 = .54$

Perfusion pressure mmHg

100
50
0

Contraction Set #4

Autoregulation to occlusion

$R_i = 117 \quad \Delta R = 60$
$R_f = 57 \quad \Delta R/R_i = 60/117 = .51$



Contraction ~45 sec

dT/dt

Tension

$T_i = 100$
$T_f = 125$

$R_i = 107 \quad \Delta R = 62$
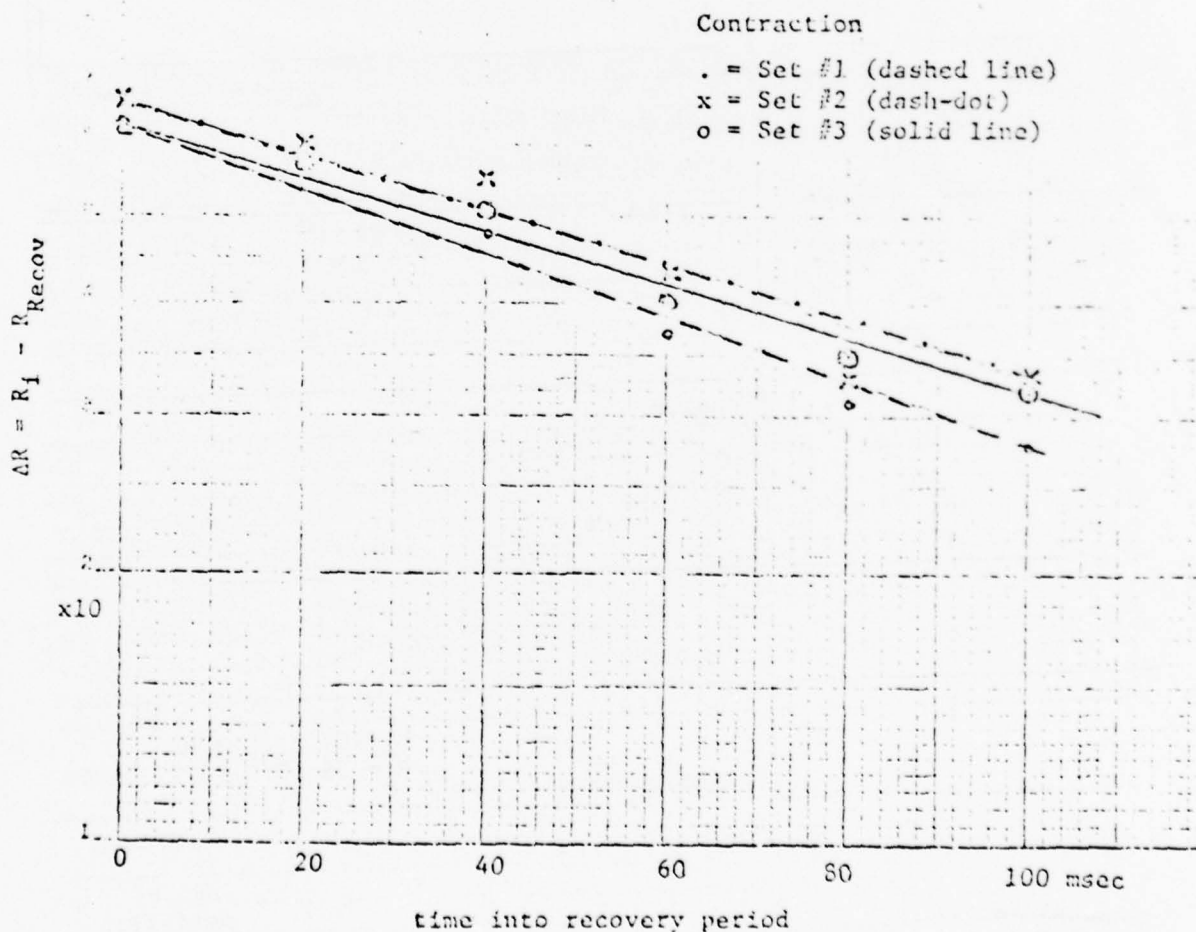$R_f = 55 \quad \Delta R/R_i = 62/107 = .58$

Perfusion pressure mmHg

last of
perfusion
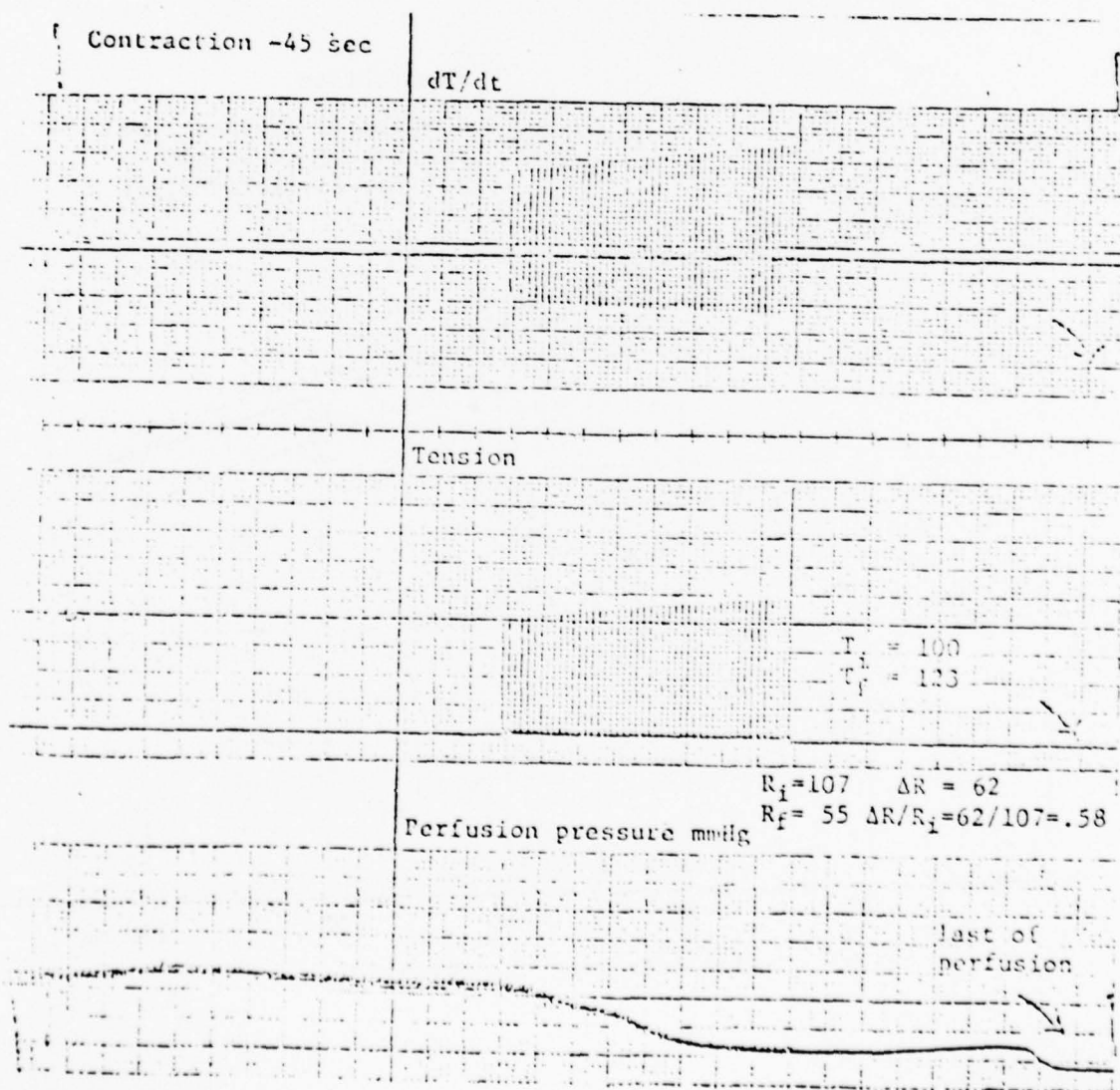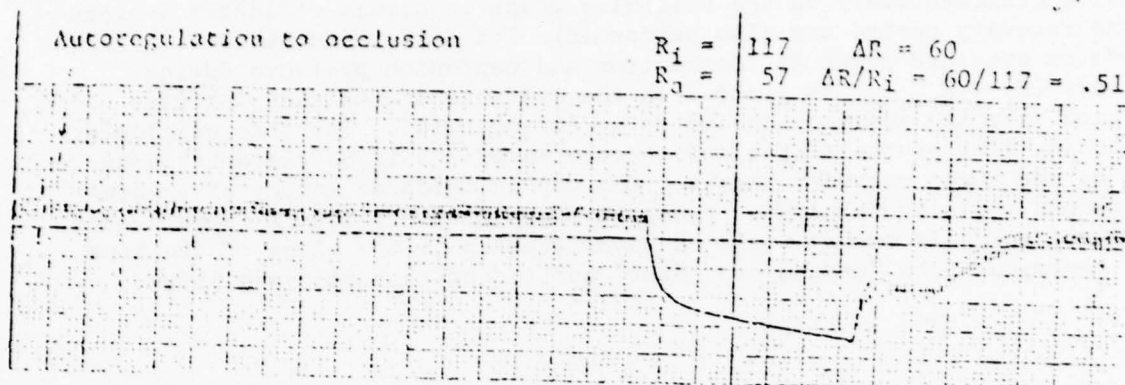
## Recovery

Initial recovery phase after each of these sets of contractions is
analyzed quantitatively on the following graph (analysis of longer periods
of the recovery period are also performed). The difference between
perfusion pressure prior to contraction and perfusion pressure during
recovery ($R_i - R_{recov}$) is plotted on the ordinate of the semi-log plot
and time into the recovery period is on the abscissa. For this particular
graph, the line of the slopes and intercepts of the lines characterizing
each of the three recovery phases. One way in which an effect of oxygen-
hemoglobin affinity on oxygen transport might manifest itself (should such
an effect actually occur) would be an alteration in the slope of the line
which represents the "oxygen dependent" portion of this recovery phase.

Recovery of Resistance after Contraction

Contraction

. = Set #1 (dashed line)
x = Set #2 (dash-dot)
o = Set #3 (solid line)

$\Delta R = R_i - R_{Recov}$

x10

time into recovery period

(Control runs have also been performed in which blood is treated in a manner identical to that employed when potassium cyanate is used to produce an in vivo curve shift, identical except for the absence of the potassium cyanate treatment itself. Blood is centrifuged, the plasma and the white cell layers suctioned off separately, and the cells washed three separate times in a solution containing 130 millimolar sodium chloride, 2.7 millimolar potassium chloride, 10 millimolar glucose and 20 millimolar TES buffer [290 milliosmols, pH 7.4] before being resuspended in plasma with the appropriate adjustment of pH and hematocrit. In control runs using this perfusate, 3 separate sets of contraction have not proven to be as reproducible as those obtained when untreated blood is used. These results are preliminary and are under further investigation. It is conceivable that the centrifugation and decanting which accompanies each of the washings is not as complete as it should be.

(iii) In vitro modification of oxygen-hemoglobin affinity--Acid citrate-dextrose (ACD) and sodium metabisulfite were used initially, as outlined in the original proposal, but each has its own associated problems. Three other methods in various stages of development now appear more promising.

ACD treated cells are depleted of 2,3-DPG, and oxygen-hemoglobin affinity is consequently increased. We have found that these treated cells, even when in ACD for only one week (ATP levels remain relatively high), and when washed and resuspended in plasma, prove to be a poor perfusate. Perfusion pressure rises rapidly and muscle performance falls off concomitantly suggesting that red cells or cell fragments may be occluding the microcirculation. Phase contrast microscopy of ACD treated cells, both before and after organ perfusion, reveal 1-2+ morphologic changes and 1-2+ rouleaux formation consistent with this hypothesis.

Incubation of red cells with sodium metabisulfite, in our laboratory, produces decreases in 2,3-DPG and $P_{50}$ comparable to those reported by others. In order to get changes of an adequate magnitude (6-8mmHg of $P_{50}$) requires 3-4 hours of incubation, which is too long a time for the requirements of our experiments.

Potassium cyanate incubation also induces significant decreases in $P_{50}$ std (10mmHg, with no accompanying change in 2,3-DPG) but in contrast to those induced by metabisulfite treatment, these require only two hours. Cyanate treated cells have normal morphology and do not cause elevation of perfusion pressure. Preliminary results indicated that changes in oxygen-hemoglobin affinity induced by potassium cyanate did adversely affect tissue oxygenation during contraction, but adequate controls are only now being obtained, as described in an earlier section. We have been assured by Dr. Anthony Cerami of the Rockefeller University (personal communication) that the exchange of cyanate from treated cells to muscle tissue is insignificant and cannot explain these results.

A contemplated intervention, not included in the original proposal, is the use of carbon monoxide. Carbon monoxide, in addition to decreasing hemoglobin-oxygen capacity, induces a leftward shift in the oxygen-hemoglobin dissociation curve. By administering carbon mor .yde at 400 parts per million to a donor dog (via an endotracheal tube) and by ventilating with a respirator at 3 liters per minute over 3 hours, we anticipate that blood with 25% carboxyhemoglobin can be obtained, blood with a 7 or 8 mm leftward shift in the oxygen hemoglobin dissociation curve. The cells from a donor dog treated in this fashion can be resuspended in pooled plasma (plasma from donor and recipient--cells from recipient also suspended in pooled plasma) to give a final hematocrit which is 4/3 that of the untreated blood. In this manner, perfusates can be obtained consisting of cells from donor (left shifted by carbon monoxide treatment) and recipient (normal $P_{50}$) suspended in the same plasma and with the same oxygen capacity, but differing in oxygen hemoglobin affinity. With suitable controls, inherent differences in the cells of the donor and recipient can be accounted for.

A final intervention which is being evaluated is the use of donor sheep blood to perfuse a recipient dog gracilis. There are sheep with naturally differing oxygen hemoglobin affinities (range 13-37mmHg) and the effects of cells from two donors with widely differing affinities suspended in pooled plasma at an appropriate hematocrit could be compared. With the cooperation of the Rochester Zoo, blood from Mufflon sheep has been obtained. the $P_{50}$ standard determined in our laboratory is 24 mmHg virtually identical to that described in the literature (23 mmHg). Dog gracilis muscle performed well, in pilot studies, when the Mufflon blood was used as perfusate. Similar studies using other sheep species are planned. and in vitro studies of the sheep blood are underway to determine red cell pH, intracellular vs. extracellular pH gradient, Bohr coefficient, temperature coefficient for converting $P_{50}$ standard to $P_{50}$ in vivo, and red cell DPG and ATP--information necessary for proper evaluation of this intervention. Similar in vitro studies involving normal dog blood and hemoglobin, and cyanated dog blood and hemoglobin are also underway.

16. Effect of hemodialysis on intraerythrocytic phosphate compounds and oxygen binding to hemoglobin.

We have studied the effects of shifts in pH and plasma inorganic phosphate $P_i$ on subjects undergoing hemodialysis. Plasma inorganic phosphate concentration fell significantly during the period of dialysis, although red cell inorganic phosphate was not influenced by this reduction (Table 1). The uptake of inorganic phosphate by the red cell was shown to be relatively rapid. Intravenous infusion of inorganic phosphate salts to a subject with chronic hypophosphatemia resulted in a proportional and prompt increase in red cell $P_i$. Red cell 2,3-DPG and ATP did not change significantly in six hours.

Exodus of inorganic phosphate from the red cell is probably very slow. This was confirmed by our studies. Normal human red cells were placed in a phosphate-free, but otherwise physiologic, salt solution containing 0.5 g/100 ml of human albumin for six hours. Intracellular $P_i$ did not fall during this period of observation.

Red Cell 2,3-DPG content was not altered by six hours of hemodialysis. Red cell ATP was also not changed by hemodialysis. Since the distribution ratio of plasma to red cell inorganic phosphate concentration expressed as $\mu$moles/ml $H_2O$ did not fall below unity during dialysis and the exodus of inorganic phosphate is slow, red cell inorganic phosphate content is not threatened by dialytic therapy as currently performed.

Plasma pH was increased during six hours of hemodialysis. Blood base changed from a slight average deficit to a moderate average excess. The net effect of the rise in blood pH and development of base excess was a slight increase in oxygen binding by hemoglobin (Figure 1). The increase in oxygen binding was modest as indicated by a mean decrement in $P_{50}$ in vivo of 0.9 torr. The increase in oxygen binding by hemoglobin was not associated with postdialysis symptoms. Two subjects had symptoms of headache and/or nausea at the termination of dialysis. In one, $P_{50}$ in vivo decreased by 1.0 torr; in another, $P_{50}$ in vivo increased by 0.5 torr. Four subjects with the largest decrease in $P_{50}$ in vivo did not have late or postdialysis symptoms (Figure 2).

17. Red cell adenosine triphosphate in hypoproliferative anemia with and without chronic renal disease: Relationship to hemoglobin deficit and plasma inorganic phosphate.

We have found that red cell ATP content, known to be elevated in subjects with anemia of chronic renal disease, was elevated, also, in the red cells of subjects with hypoproliferative anemia without renal disease. In anemic subjects, with and without renal disease, the increase in red cell ATP was associated with the extent of hemoglobin deficit; however, the increment in red cell ATP was greater in subjects with chronic renal disease at a given reduction in hemoglobin concentration. In subjects with chronic renal disease, red cell ATP content was also strongly correlated with plasma inorganic phosphate ($P_i$) concentration. The latter relationship appeared to explain the additional increase in red cell ATP, although these studies do not allow conclusions as to causality. Normal and

reduced plasma $P_i$ concentrations were associated with a reduced red cell
ATP content for a given hemoglobin deficit in subjects with chronic renal
disease. Red cell magnesium was elevated in subjects with chronic renal
disease and in subjects with hypoproliferative anemia. Reticulocyte ATP
was three-fold the mean population ATP concentration in normal subjects.
This difference fits an exponential decay in red cell ATP with aging. It
is possible therefore, that age-dependent hemolysis may explain population
red cell ATP content in hypoproliferative anemias. Selective changes in
the age of red cell populations may explain the quantitative variation in
mean red cell ATP levels (Figure 3).

18. Effects of contrast materials on red cell membrane potential and
plasma and red cell pH.

In these studies, contrast materials have been shown to produce
an acute reduction in blood pH. By adding impenetrable organic anions to
the external milieu, the electrical effect of internal red cell organic
anions is counteracted and the negative potential across the red cell
membrane is nullified. The red cell membrane potential (E) expressed as
volts may be calculated from the equilibrium distribution of chloride by
use of the Nernst equation:

$$E = -\left[ \left( \frac{RT}{zF} \right) \quad \ln \quad \frac{[Cl^-]_e}{[Cl^-]_i} \right]$$

where R, the gas constant, equals 8.314 joule/°K per mole; T, the absolute
temperature, is 310°K; z, the valence of the chloride ion, is unity; F, the
Faraday constant, is 96493 coulomb/eq; and $[Cl^-]$ is the concentration of
chloride in plasma water (e) and in cell water (i). The activity coefficient
of $Cl^-$ is assumed to be the same in cytosol as in plasma.

The anion equivalency of impenetrable compounds in the red cell
is approximately 70 mEq/L $H_2O$. This total is composed of hemoglobin ($\sim$ 40
mEq/L), 2,3-diphosphoglycerate ($\sim$ 20 mEq/L), adenosine triphosphate ($\sim$ 5
mEq/L) and other phosphates ($\sim$ 5 mEq/L). If the effect of contrast material
is due to its balancing, the internal impenetrable anions, the membrane
potential should be obliterated when approximately 70 mEq/L $H_2O$ of sodium
Hypaque is added to the external milieu.

In order to test this hypothesis, we prepared isotonic sodium
Hypaque solutions and added isotonic sodium chloride so as to achieve
solutions of 0 to 135 mM sodium Hypaque which were isotonic (280-300 mOsm).
Since 1 mM sodium Hypaque produced 2 mOsm, we assumed complete dissociation
of the compound in solution. We, thereafter, titrated the equivalents of
extracellular Hypaque anion required to reduce the membrane potential to
zero and beyond, as shown in Table 5. Addition of Hypaque resulted in a
reduction and finally inversion of the membrane potential as increasing
concentrations of Hypaque were added. By extrapolation, the membrane
potential was zero at $\sim$ 65 mEq/L of Hypaque which is very close to the
expected average value of 70 mEq/L (Table 2). This reduction in negativity
of the inside with respect to the outside of the membrane allows the rate
of influx of permeable anions, hydroxyl, bicarbonate and chloride to increase
and equal or exceed that of efflux. The greater buffering capacity of the
interior of the red cell and the increase in $Pco_2$ in the

red cell prevent a significant change in internal pH, whereas plasma is acidified by the net increase in hydrogen ion concentration which occurs.

Coronary sinus blood was sampled following injection of Renografin or Hypaque into the coronary artery. pH measured in blood collected 5 to 8 seconds after dye injection decreased in each of four dogs studied (Figure 4). Red cell pH did not change significantly (not shown). The fall in pH was closely associated with the concentration of dye present in the blood samples studied. The magnitude of the pH fall observed was greater than that in renal blood, due to the ability to sample more rapidly. If 0.5 M $NaHCO_3$ was added to Hypaque solutions prior to injection, the fall in plasma pH was prevented, although in this case red cell pH increased significantly (e.g., 7.19 to 7.25). The abrupt change in plasma pH could contribute to the abnormality of membrane and cell function, which may occur following injection of contrast material, especially into the coronary circulation.

19. The use of a single venous blood sample to measure the in vivo $P_{50}$ of hemoglobin.

The affinity of hemoglobin for oxygen is of clinical importance because it may decrease in response to hypoxia, anemia or reduced blood flow and acts, thereby, to maintain venous (i.e. tissue) $PO_2$ as oxygen extraction increases. In situations in which the oxygen-hemoglobin equilibrium is altered as a consequence of deficits in oxygen content or flow, the effect of the four major determinants of the equilibrium, that is red cell, 2,3-DPG, pH, temperature, and $Pco_2$ must be considered. This has been called the oxygen-hemoglobin dissociaton curve at in vivo conditions and is represented by the $P_{50}$ in vivo ($P_{50}$ iv).

An assessment of the affinity of hemoglobin for oxygen is considered inaccessible to the practicing physician and hematologist since it requires tonometry and mixing techniques often available only in research laboratories. In the following studies, we examined the usefulness of a single venous blood sample as an indicator of the position of the oxygen-hemoglobin dissociation curve. A single venous blood sample, analyzed for pH, $PO_2$ and $SO_2$ by a clinical laboratory could be used by a physician to assess the presence of an alteration in the oxygen-hemoglobin equilibrium.

Venous blood samples were obtained from healthy subjects as well as those with hypoproliferative anemia, congestive heart failure, ischemic heart disease, acidosis, alkalosis, sickle cell disease and from umbilical cord blood.

The CALCULATED (CALC) $P_{50}$ iv of venous blood at 37°C was determined from the formula:

$$CALC\ P_{50}iv = antilog\ (log\ P_{50}\ std + 0.48\ (7.40-pH) + 0.0013\ (BE))$$

where $P_{50}$ std was derived for a hemoglobin-oxygen dissociation curve.

The AS RECEIVED (AS REC) $P_{50}$ iv was determined from the formula:

$$AS\ REC\ P_{50}\ iv = antilog\left[\frac{\log 1/k}{n}\right]$$

where

$$1/k = antilog\ (n\ \log PO_2)\ X\left[\frac{100-SO_2}{SO_2}\right]$$

$PO_2$ and $SO_2$ were measured in the venous blood. The Hill Constant, n, was considered to be 2.7 in all subjects. If necessary, AS REC $P_{50}$ iv can be adjusted for body temperature by the formula:

$$P_{50}\ iv = antilog\ [\log P_{50} + 0.024\ (t-37°C)]$$

where t is subject's body temperature at the time of sampling. All formulae were entered into a Wang 600 programmable calculator.

Figure 5 depicts the relationship in 102 subjects between AS REC $P_{50}$ iv at 37°C determined by a single $PO_2$ and $SO_2$ measured in venous blood and the CALC $P_{50}$ iv at 37°C determined by, firstly, preparing a three to five point oxygen-hemoglobin dissociation curve, secondly, deriving the $P_{50}$ std from the curve and, thirdly, adjusting the $P_{50}$ std to the pH and base excess of the venous blood at the time of sampling. A strong and highly significant correlation was present (r = 0.8, P < 0.001). Moreover, the slope of the curve was such that AS REC $P_{50}$ iv was in excellent agreement with CALC $P_{50}$ iv above a $P_{50}$ of 26 torr and was only slightly greater (maximally 0.5 torr) than CALC $P_{50}$ iv as $P_{50}$ decrease from 26 to 21 torr.

Eleven subjects with either alkalosis or acidosis were studied also. CALC $P_{50}$ iv derived from the $P_{50}$ std, pH and base excess or deficit was very similar to the AS REC $P_{50}$ iv based on a single venous blood sample. The correlation coefficient for the association of CALC $P_{50}$ iv with AS REC $P_{50}$ iv determined from a single venous blood sample was very high (r = 0.9, P < 0.001).

An important question regarding the AS REC $P_{50}$ iv is the validity of using venous rather than arterial blood to assess $P_{50}$ iv. Since $P_{50}$ std is the same in venous and arterial blood, the major concern is the difference in pH of venous as compared to arterial blood. We, as others, have found that arterial pH is very closely correlated with venous pH. Figure 6 depicts the relationship of central venous to arterial pH based on 170 observations from arterial and venous blood samples measured simultaneously. A very high correlation (r = 0.93, P < 0.001) was present. Mean venous pH was about 0.03 pH units less than arterial at an arterial pH of 7.40.

In situations where tissue lactate production is very high, as in shock, use of venous blood to measure $P_{50}$ _in vivo_ may be misleading since red cell pH would not be influenced by blood lactate during the time required for capillary transit. Since $CO_2$ rapidly enters the red cell and is hydrated instantaneously by the action of red cell carbonic anhydrase, increases in $P_{CO_2}$ as blood traverses tissue capillaries can produce changes in oxygen-binding to hemoglobin that may be functionally important. Under all but the most extreme circumstances the pH gradient between artery and vein is related to differences in $P_{CO_2}$. Indeed, in attempting to quantify the effect role of oxygen binding to hemoglobin on oxygen delivery the $P_{50}$ gradient from artery to vein may be the most valid estimate, excluding the effect of excess lactate. Central rather than peripheral venous blood is most useful for measuring $P_{50}$ _in vivo_.

20. _The use of a single venous blood sample to measure $P_{50}$ at standard conditions so as to detect mutant hemoglobins._

Also, measuring the strength of oxygen binding to hemoglobin at standard _in vitro_ conditions is of clinical importance because it is a means of detecting the presence of a mutant hemoglobin with high or low affinity for oxygen. Hemoglobins with markedly altered affinity for oxygen may be the cause of polycythemia or, less commonly, anemia.

The evaluation of patients with polycythemia is hampered by the requirement for special equipment to do oxygen-hemoglobin dissociation curves. These instruments, found in a few laboratories, are often inaccessible to the practicing physician. We show here that measurement of venous blood, pH, oxygen tension ($P_{O_2}$), and oxygen saturation ($S_{O_2}$), as performed in a clinical chemistry laboratory, is a useful means for detecting hemoglobin with an altered affinity for oxygen.

$$AS \ REC \ P_{50} \ std = antilog \ \frac{log \ 1/k}{n} \ ,$$

$$where \ 1/k = [antilog \ (n \ log \ P_{O_2(7.4)})] \cdot \frac{100 - S_{O_2}}{S_{O_2}}$$

A Hill constant (n) for hemoglobin A of 2.7 was used in all calculations. The $P_{O_2}$ in venous blood, measured at 37°C was converted to $P_{O_2}$ at pH 7.4 with the formula:

$$log \ P_{O_2(7.4)} = log \ P_{O_2} - [0.5 \ (7.40 - pH)],$$

where pH represents the value in the antecubital venous blood.

The $P_{O_2(7.4)}$ and $S_{O_2}$ of antecubital venous blood from 38 healthy subjects are shown in Figure 7A. These observations fall near the curve expected for the oxygen-hemoglobin equilibrium of normal blood. The shaded area in Figure 7B depicts the range for healthy subjects. The $P_{O_2(7.4)}$ and $S_{O_2}$ of antecubital venous blood from healthy subjects with polycythemia vera and hypoxic polycythemia fall within the range for healthy subjects. The $P_{O_2(7.4)}$ and $S_{O_2}$ of the antecubital venous blood from subjects with structural variants of hemoglobin with altered oxygen affinity fall outside the range for healthy subjects.

The distribution of $P_{50}$ calculated from the venous $Po_{2(7.4)}$ and $So_2$ is shown in Figure 8. The blood of healthy subjects had a mean $P_{50}$ std of 26 ± 1.3 (S.D.) mm Hg. The 99% confidence interval for individual observations was 22.6 to 29.4 mm Hg. The $P_{50}$'s derived from six observations in three subjects with hemoglobin Bethesda and a single observation in subjects with either hemoglobin's Olympia, Rainier, or Yakima, all high-affinity hemoglobins, were outside the 99% lower confidence limit for healthy subjects. Also, two observations in one subject with >90% hemoglobin S, who was known to have erythrocytes with low-oxygen affinity from prior studies of her oxygen-hemoglobin dissociation curve, were far outside the upper confidence limits for healthy subjects.

The AS REC $P_{50}$ std of subjects with hemoglobin Bethesda (∿ 15 mm Hg) was somewhat higher than the $P_{50}$ std measured with a full oxygen-hemoglobin dissociation curve (∿ 9 mm Hg). This was explained by the profound deviation of the oxygen-hemoglobin dissociation curve of this mutant hemoglobin from the sigmoid curve that is described mathematically by the Hill equation. Nevertheless, the $P_{50}$ std was markedly abnormal and detected the oxygen binding abnormality unequivocally. In subjects with hemoglobin's Olympia, Rainier, Yakima, and S, the AS RECEIVED $P_{50}$ std was within 1 mm Hg of the $P_{50}$ obtained from a full oxygen hemoglobin dissociation curve. In previous studies, a close correlation of AS RECEIVED $P_{50}$ std with $P_{50}$ std derived from a full curve in subjects with normal hemoglobin was found.

21. The role of hemoglobin-oxygen affinity in oxygen transport to ischemic myocardium.

Fourteen patients, ten with angina and four with atypical chest pain were studied during diagnostic cardiac catherization and coronary angiography. An attempt was made to re-examine the suggestion that a decrease in hemoglobin-oxygen binding occurs across the coronary bed during pacing induced angina. It has been further suggested that this decrease is not explained by any of the known determinants of $P_{50}$. We were not able to confirm these observations that had been reported from another laboratory. Utilizing careful paired sample analyses, we could not detect any changes during pacing-across the coronary circulation-- in $P_{50}$ measured at standard conditions (pH 7.4, $Pco_2$ 40 torr, T 37°C), or in red cell 2,3-DPG level. What we did find was that patients developed respiratory alkalosis during pacing. This alkalosis was sufficient to produce a significant reduction in $P_{50}$ at in vivo conditions of pH. There was an average fall in $P_{50}$ of 1.6 torr, and a decrease of 3.0 torr was observed in one patient. Thus, a significant increase in hemoglobin-oxygen binding was observed. Patients with coronary disease, and those with normal coronaries all became alkalotic. The mechanism appeared to be a hyperventilatory response to pacing since a consistent fall in $P_a CO_2$ accompanied the alkalosis. If hyperventilation and respiratory alkalosis occur during spontaneous angina, then in regions of the coronary vascular bed where flow is fixed, the fall in $P_{50}$ might be deleterious to oxygen release and compound the limitation in oxygen transport caused by restricted blood flow.

22. The role of hemoglobin-oxygen affinity in oxygen transport during congestive heart failure.

Twenty two patients with varying degrees of chronic cardiac decompensation were studied during diagnostic cardiac catherization. The interrelationships among arterial oxygen flow rate ($OFI_A$), oxygen binding by hemoglobin and whole body oxygen utilization were examined. Despite a reduction of 63% in systemic oxygen transport from the highest to the lowest $OFI_A$, oxygen consumption was relatively well maintained because there was an increase in proportional extraction of oxygen, in close association with falling $OFI_A$. There was also an increase in $P_{50}$, both at standard and at in vivo conditions as $OFI_A$ fell, and rising proportional extraction and $P_{50}$ were significantly correlated with one another (r=0.50). We calculate that about one-third of the increase in proportional extraction of oxygen that was observed, as $OFI_A$ fell, could be explained by rising $P_{50}$, that is--by a decrease in hemoglobin affinity for oxygen in tissue capillaries. Thus, altered hemoglobin oxygen affinity appears to be an important adaptive mechanism for maintaining tissue oxygen utilization when systemic oxygen transport is impaired in patients with chronic heart disease.

23 · Gracilis muscle model for studies of oxygen transport.

We have developed an isolated muscle model to test the hypothesis that altered hemoglobin-oxygen binding can influence tissue oxygen uptake when blood flow and arterial blood oxygen content are held constant (Figure 9). The model is a variation of that described by Renkin in Acta Physiol. Scand. 54: 223, 1962.

In our initial experiments, the dog gracilis muscles were isolated, the gracilis artery and vein were cannulated and the muscle was perfused with blood that had been collected earlier the same day from the same dog. The blood had been treated in one of several ways to modify $Hb-O_2$ affinity, then oxygenated and passed through a finger pump into the muscle. Various blood treatment modalities were tested, including (1) blood storage in ACD to reduce 2,3-DPG levels, (2) exposure to metabisulfite (3) treatment with potassium cyanate to carbamylate the hemoglobin. All such treatments appeared to produce red cell damage and perhaps sludging so that muscle vascular resistance rose dramatically during blood infusion, and interpretation of the data was difficult. These manipulations will, nevertheless be pursued, as these findings may have an important bearing on oxygen transport when patients are transfused with stored blood.

At present, however, we are autoinfusing the muscle from the donor dog and are manipulating hemoglobin-oxygen affinity by inducing respiratory alkalosis (Bohr effect). In a typical experiment, the following protocol is followed after muscle isolation and establishment of controlled flow:

1. Control gracilis arterial (A) and venous (V) blood sampling
2. Induction of respiratory alkalosis by hyperventilation
3. Repeat A, V sampling for determination of resting muscle $\dot{V}O_2$ and lactate production
4. Stimulation of the muscle for approximately one minute and measurement of $\dot{V}O_2$ and lactate production during exercise
5. Collection of blood samples during recovery period
6. Restoration of ventilation to normal is followed by a period to allow recovery of muscle to basal conditions
7. Repeat stage 3-5

8. Alteration of blood flow rate to the muscle and repeat of steps 3-7

9. At each step blood samples are taken for determination of blood pH, $PO_2$, $Pco_2$, %$HbO_2$, %$HbCO$, $Hb$ level, lactate concentration. Such sampling allows not only calculation of $\dot{V}O_2$ and lactate production but of $P_{50}$ at both standard and _in vivo_ conditions

10. At each step, the muscle is also subjected to a test for arterial occlusion to be sure that it has retained its capacity to autoregulate and is thus behaving physiologically.

The preliminary data suggest that in the range of normal flow rates, alkalosis does not impair either resting or exercise $\dot{V}O_2$ in spite of the associated reduction in $P_{50}$ _in vivo_. However, during alkalosis, the muscle appears to operate at lower levels of venous $PO_2$. It is possible, then that with further reduction in arterial flow rate, or with more extreme exercise, induced affinity changes may have an impact on tissue $O_2$ uptake.

**Table 1.** Effect of hemodialysis on red cell and plasma inorganic phosphorus

|  | Predialysis | Postdialysis |
|---|---|---|
| Plasma $P_i$ | 2.01 ± 0.32 | 1.14 ± 0.19 |
| Red cell $P_i$ | 0.58 ± 0.093 | 0.57 ± 0.098 |

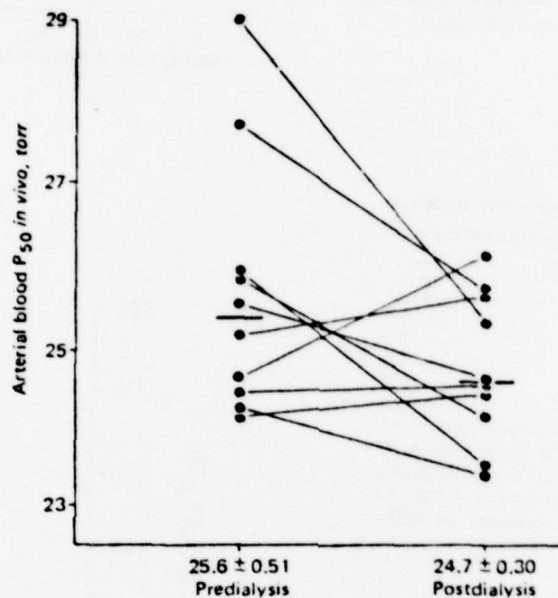Values represent the mean ± SEM of μmoles/ml of plasma or cells.



**Fig. 1** *Arterial blood $P_{50}$ in vivo before and after hemodialysis.* The horizontal bar represents the median value. The mean ± SEM is also shown below.
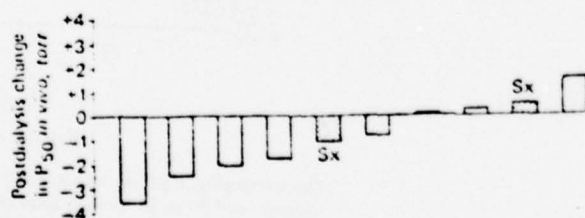


**Fig. 2** *The change in $P_{50}$ in vivo after six hours of dialysis is shown for ten subjects studied.* Two subjects had late dialysis symptoms (Sx) of headache and/or nausea.

Fig 3 The mean red cell ATP content of red cell populations according to the population life-span. The arrows indicate the mean red cell ATP for anemic subjects with and without chronic renal disease. If age-dependent shortening of survival explains the elevated red cell ATP content, the survival of red cells would be 50 and 80 days for those with and without chronic renal disease respectively. The variation in red cell ATP in subjects with anemia                    4) would, in part, be explained by the relative contributions of age-dependent hemolysis in each subject
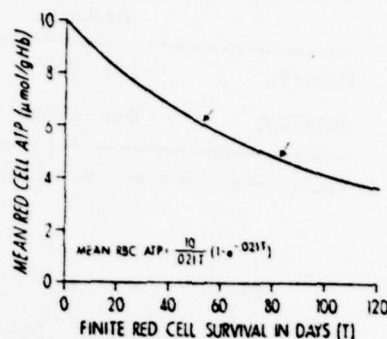


$$\text{MEAN RBC ATP} = \frac{10}{0.211} (1 - e^{-0.211 T})$$

## Table 2

*Red Cell Membrane Potential (E) in the Presence of Hypaque*

| Hypaque concentration (M) | $\ln \frac{[Cl^-]_o}{[Cl^-]_i}$ | E (mV) |
|---|---|---|
| 0.0 | +0.48 | − 12.8 |
| 0.045 | +0.14 | − 3.7 |
| 0.075 | − 0.07 | + 1.9 |
| 0.090 | − 0.30 | + 8.0 |
| 0.135 | − 1.1 | +29.4 |

Final concentration in solution was 0.150 M in each case. NaCl concentration was equal to 0.150 M minus the Hypaque concentration. E represents potential of the inside of red cell membrane with respect to the outside expressed in millivolts. $E (mV) = -26.7 \ln \frac{[Cl^-]_o}{[Cl^-]_i}$.
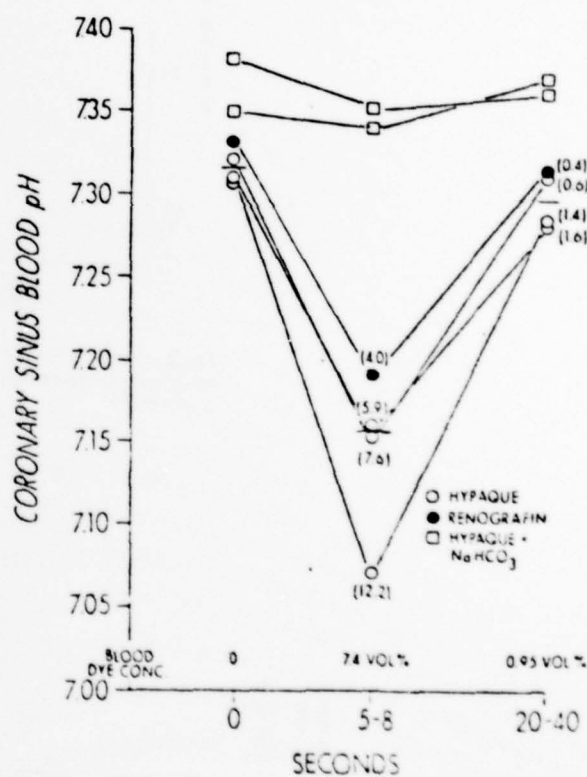


Figure 4

The extracellular pH in dog coronary sinus blood before, 5 to 8 seconds, and 20 to 40 seconds after injection of Hypaque (unfilled circles) or Renografin (filled circles). The mean blood contrast material concentration is shown in ml contrast/100 ml blood (vol %). The unfilled squares represent the blood pH when Hypaque to which 0.5 M NaHCO₃ was added was injected into the coronary artery. The parentheses contain the concentration of contrast material in the blood.
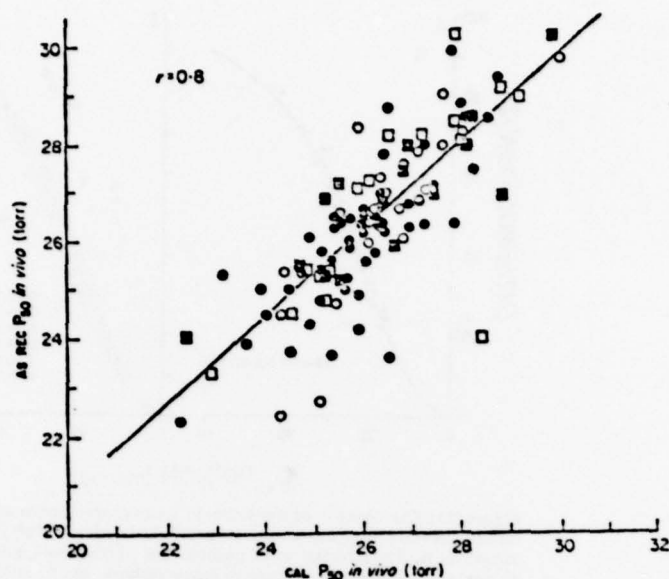
FIG 5 The association of AS REC $P_{50}$iv with CALC $P_{50}$iv in healthy subjects (○) and subjects with hypoproliferative (reticulocytopenic) anaemia (□), ischaemic heart disease (●) and congestive heart failure (■) is shown. AS REC $P_{50}$iv = 0.91 (CALC $P_{50}$iv) + 2.60.
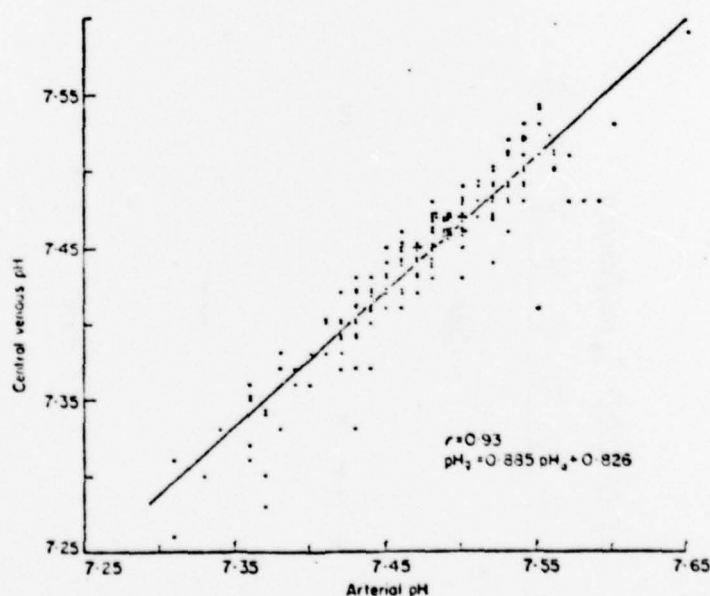


FIG 6 The relationship of central venous pH to arterial pH is depicted. 170 observations in 62 subjects are included.
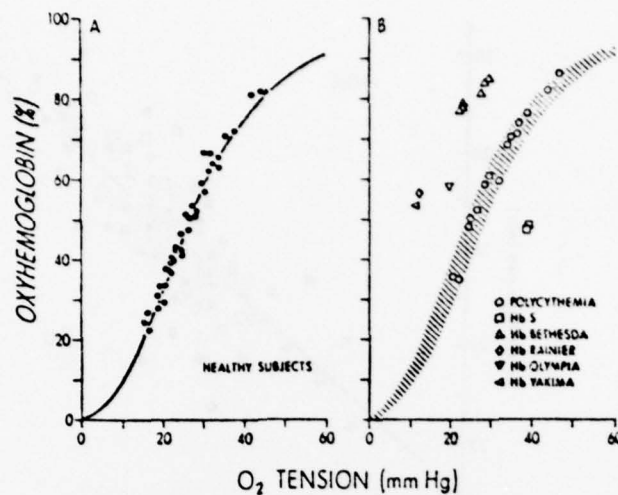
**Figure 7A.** The percent of hemoglobin as oxyhemoglobin at the ante-cubital venous Po₂, corrected to pH 7.4, is shown for 38 healthy subjects. **B.** The shaded area depicts the normal range for healthy subjects. The circles represent observations in 5 subjects with hypoxic polycythemia and 10 with polycythemia vera. The triangles, squares, and diamonds represent observations in subjects with mutant hemoglobins with altered oxygen affinity.
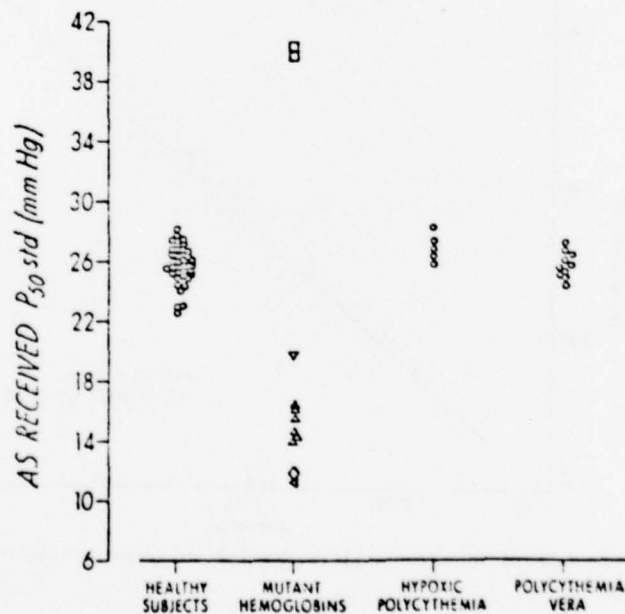


**Figure 8** The AS REC P₅₀ std's calculated from the antecubital venous blood pH, Po₂, and So₂ are shown for healthy and poly-cythemic subjects and those with mutant hemoglobins. The symbols for subjects with mutant hemoglobins correspond to those in Figure 7B.

CONTROL  EXPERIMENTAL

STIRRING MOTOR

LOOD INLET/FILTER

PADDLE

(C)
)UAL IL
RESERVOIR

FILTER

(D)
OLLOW
IBRE
XYGENATOR

(E)
PH STAT

(A)

NSULATED
OX, TWO
OMPARTMENT

TWIN
PUMP

PRESSURE
MONITOR

(B)

SOLENOID SHUT-
OFF VALVE

GRACILIS
ARTERY

GRACILIS
VEIN

TO STRAI
GAUGE

NERVE

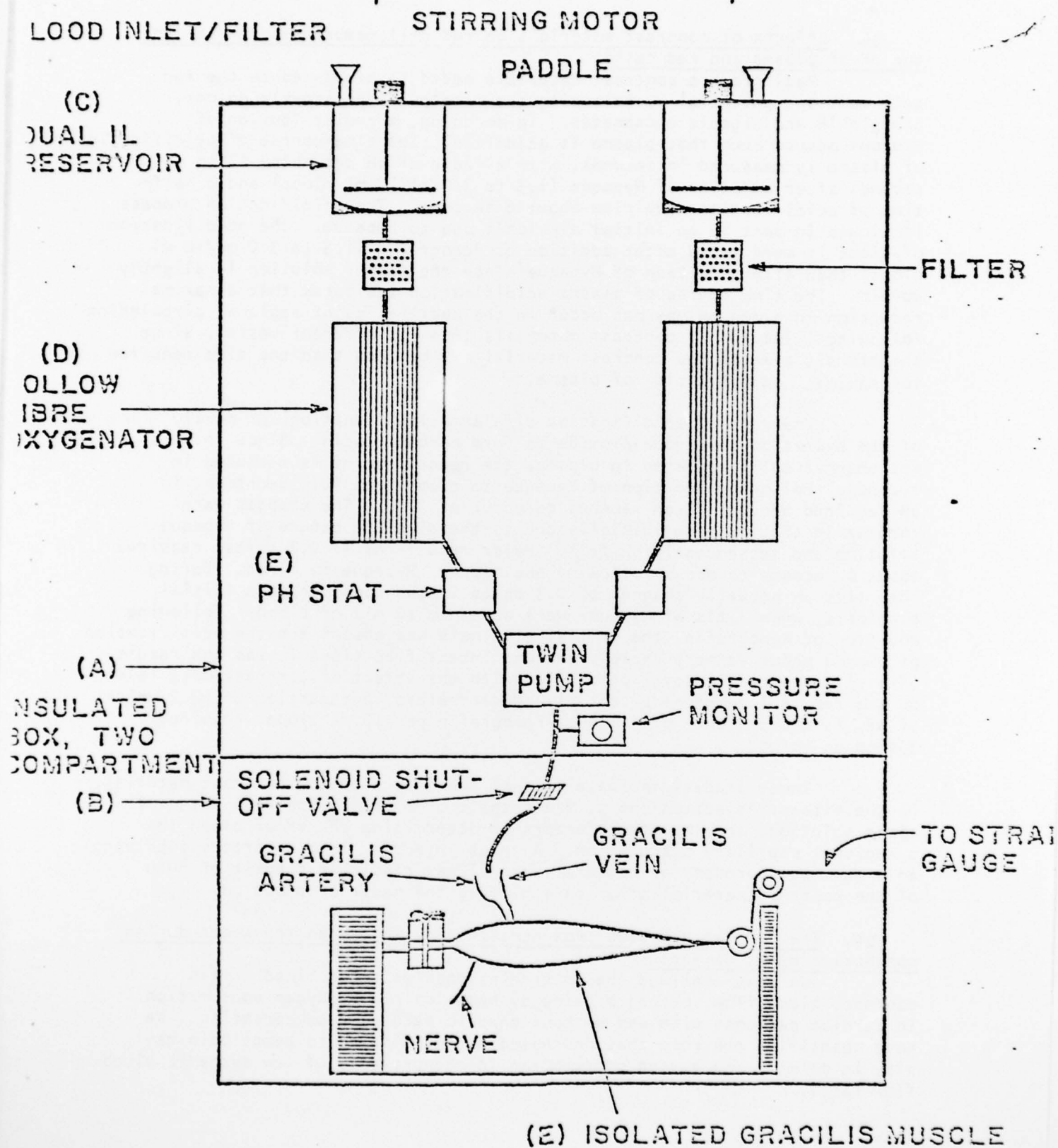(E) ISOLATED GRACILIS MUSCLE

Figure 9

24.  Effects of contrast materials on red cell membrane potential and the pH of plasma and red cells.

Radiographic contrast materials added to blood reduce the red cell membrane potential by balancing the internal impenetrable anions, hemoglobin and organic phosphates.  In so doing, a redistribution of protons occurs such that plasma is acidified.  The time course of acidification of plasma is measured in seconds, with a nadir of pH occurring 12 to 15 seconds after addition of Hypaque (1.5 to 3.0 ml/10 ml blood) and a half-time of acidification requiring about 6 seconds.  The acidification process is slowed in part by an initial alkalosis due to Hypaque.  The acidification of blood is more rapid after addition of Renografin (1.5 to 3.0 ml/10 ml blood) than after addition of Hypaque since the former solution is slightly acidic.  The time course of plasma acidification indicates that a maximal reduction in blood pH may not occur in the capillaries of regional circulation following injection of contrast materials into its afferent vessel, since the transit time of the contrast material may be less than the time required for maximal  acidification of plasma.

The rate of acidification of plasma is a function, primarily of the hydration of carbon dioxide to form carbonic acid.  Since this is not enzymatically mediated in plasma, the reaction time is measured in seconds.  Following addition of Hypaque to blood, the full decrement in pH required about fifteen seconds to occur at 37°C.  The changes were complex in that pH rose initially due to the alkaline nature of Hypaque solution and subsequently pH fell.  Major reductions (> 0.2 units) required about 6 seconds to occur following addition of Hypaque to blood.  During this time pH actually changed by 0.3 units if one considers the initial alkalosis, when 3 mls of Hypaque were added to 10 mls of blood.  Following addition of Renografin, the initial alkalosis was absent and the acidification of plasma occurred more rapidly and was intensified since it was the result of a slightly acidic solution coupled with the effect of contrast materials on the red cell membrane potential.  Nevertheless, a reduction of >0.2 units of pH, in the presence of 3 mls of Renografin per 10 ml blood, required 3 seconds to occur.

These studies indicate that a) the nature of the contrast material, b) the site of injection and c) the transit time of the contrast material-blood solution, are important factors in determining the pH of blood in a regional capillary circulation.  A bolus injection into an artery supplying an organ (eg. coronary or cerebral artery) may result in transit of much of the contrast material prior to achieving the nadir of blood pH.

25.  The role of hemoglobin-oxygen affinity in oxygen transport during congestive heart failure.

We have examined the interrelationships among blood oxygen content, blood flow, oxygen binding by hemoglobin and oxygen consumption in cardiac patients with and without chronic cardiac decompensation.  We have quantified the role that decreased oxygen-binding to hemoglobin may play in maintaining oxygen consumption in the presence of low systemic blood flow rates.

The volume rate of oxygen delivery to tissues was expressed as the arterial oxygen flow rate index ($OFl_a$), the product of oxygen content and blood flow. $OFl_a$ varied from 738 to 262 mls $O_2 \cdot min^{-1} \cdot m^{2-1}$ whereas oxygen consumption ($\dot{V}_{O2}$) varied from 170 to 117 mls $O_2 \cdot min^{-1} \cdot m^{2-1}$. Thus, mean $\dot{V}_{O2}$ fell only 19% despite a mean decrease in $OFl_a$ of 63%. $\dot{V}_{O2}$ was maintained because the extraction of oxygen rose from about 20% to 50% in close association with the decrease in $OFl_a$.

Oxygen binding to hemoglobin decreased as $OFl_a$ decreased. At in vivo conditions of pH, $P_{CO2}$ and temperature, $P_{50}$ in vivo rose; this facilitation of oxygen release at the $P_{O2}$ of tissue capillaries could explain about one third of the observed increase in oxygen extraction as $OFl_a$ fell. An alternative interpretation is that an increase in $P_{50}$ in vivo minimizes the reduction in mixed venous $P_{O2}$ needed to maintain $\dot{V}_{O2}$ when increasing proportional extraction of $O_2$ compensates for decreasing $OFl_a$.

Articles published, July 1, 1973 to March 31, 1978 with support of
Contract DA17-73-C-3135

1. Lichtman. M.A. Therapeutic manipulation of hemoglobin oxygen affinity:
   Possible role in the management of acute hypoxia. Preservation of Red
   Blood Cells, National Research Council, National Academy of Sciences,
   Edit. H. Chaplin, Jr., et. al, Washington, D.C., 1973.

2. Young, J.A., Lichtman. M.A. and Cohen, J. Reduced red cell 2,3-
   diphosphoglycerate and adenosine triphosphate, hypophosphatemia and
   increased hemoglobin-oxygen affinity after cardiac surgery. Circula-
   tion 47: 1313, 1973.

3. Udkow, M., LaCelle, P.L. and Weed, R.I. The effects of pH, oxygen
   tension and 2,3-diphosphoglycerate concentration upon the binding of
   adenosine triphosphate to concentrated hemoglobin A solutions.
   Nouv. Rev. Fr. Hemat. 13: 817, 1973.

4. Lichtman, M.A., Marinetti, G.V. and Gordesky, S.R. The effect of
   incubated plasma and lysolecithin on the shape and membrane lipid
   composition of red cells studied in vitro. Nouv. Rev. Fr. Hematol.
   14: 5, 1974.

5. Lichtman, M.A., Murphy, M.S., Byer, B.J., Freeman, R.B. Hemoglobin
   affinity for oxygen in chronic renal disease: the effect of hemodialysis.
   Blood 43: 417, 1974.

6. Lichtman, M.A., Murphy, M.S., Whitbeck, A.A. and Kearney, E.A. Oxygen
   binding to hemoglobin in subjects with hypoproliferative anemia, with
   and without chronic renal disease: Role of pH. Brit. J. Haematol.
   27: 439, 1974.

7. Lichtman, M.A., Cohen, J., Murphy, M.S., Kearney, E.A. and Whitbeck, A.A.
   Effect of propranolol on oxygen binding to hemoglobin in vitro and in
   vivo. Circulation 49: 881, 1974.

8. Lichtman, M.A., Cohen, J., Young, J.A., Whitbeck, A.A. and Murphy, M.S.
   The relationship between arterial oxygen flow rate, oxygen binding by
   hemoglobin and oxygen utilization following myocardial infarction.
   J. Clin. Invest. 54: 501, 1974.

9. Lichtman, M.A., Cohen, J. and Young, J.A. Reduced arterial oxygen flow and
   respiratory alkalosis following myocardial infarction: Role of hemoglobin-
   oxygen affinity in the maintenance of oxygen consumption. Trans. Assoc.
   Amer. Phys. 87: 233, 1974.

10. Lichtman, M.A. The effect of hemodialysis on intraerythrocytic phosphate
    compounds and oxygen binding to hemoglobin. Kidney International Suppl.
    2: S-134, 1975.

11. Lichtman, M.A., Whitbeck, A.A. and Murphy, M.S. Factitious changes in
    binding of oxygen to hemoglobin when based on extracellular pH in the
    presence of certain blood additives like radiographic contrast media.
    Invest. Radiol. 10: 225, 1975.

12. Lichtman, M.A.  The effect of hemodialysis on intraerythrocytic phosphate compounds and oxygen binding to hemoglobin.  Kidney International Suppl. 2:S-134, 1975.

13. Lichtman, M.A. and Murphy, M.S.  Red cell adenosine triphosphate in hypoproliferative anemia with and without chronic renal disease: Relatinship to hemoglobin deficit and plasma inorganic phosphate (Proceedings of a conference on Stress Erythropoiesis, May, 1975, Rochester, New York) Blood Cells 1: 467, 1975.

14. Lichtman, M.A.  Does ATP decrease exponentially with red cell aging? Nouv. Rev. Franc. D'Hematol. 15: 625, 1975.

15. Lichtman, M.A. and Lipchik, E.O.  Acidification of the plasma by the red cell in the presence of radiographic contrast materials:  a possible cause of adverse effects on the heart.  Trans. Assoc. Amer. Phys. 88: 265, 1975.

16. Lichtman, M.A., Murphy, M.S., Whitbeck, A.A., Pogal, M., and Lipchik, E.O.  Acidification of plasma by the red cell due to radiographic contrast materials.  Circulation 52: 943, 1975.

17. Lichtman, M.A., Murphy, M.S., and Pogal, M.  The use of a single venous blood sample to assess oxygen binding to hemoglobin. Brit. J. Haemat.  32: 89, 1976.

18. Lichtman, M.A., Murphy, M.S., and Adamson, J.W.  A simplified technique for the detection of mutant hemoglobins with altered affinity for oxygen.  Ann. Int. Med. 84: 517, 1976.

19. Lichtman, M.A. and Murphy, M.S.  Reduced red cell membrane potential and acidification of the plasma in response to contrast materials: Time course of the alteration in plasma pH.  Invest. Radiol. 11:588, 1976.

20. Daniel, A., Cohen, J., Lichtman, M.A., Murphy, M.S., Schreiner, B.F., Jr., and Shah, P.M.  The relationships among arterial oxygen flow rate, oxygen binding by hemoglobin and oxygen utilization in chronic cardiac decompensation.  J. Lab. Clin. Med. 1:635, 1978.

21. La Celle, P. L. and Lichtman, M.A.  Anemia with reticulocytosis: Hemolytic disorders.  In:  Hematology for Practitioners.  Little, Brown and Company, Boston, 1978. p. 51.

22. Cohen, J. and Lichtman, M.A.  Polycythemia.  In:  Hematology for Practitioners. Little, Brown and Company, Boston, 1978. p. 87

23. Zesk, J., Cohen, J. and Lichtman, M.A.  Effect of altered Hb-$O_2$ binding on oxygen uptake by isolated gracilis muscle.  Circulation. 1977. (Abstract).

| 4 copies | HQDA (SGRD-SI)<br>Fort Detrick<br>Frederick, MD. 21701 |
| --- | --- |
| 12 copies | Defense Documentation Center (DDC)<br>ATTN: DDC-DCA<br>Cameron Station<br>Alexandria, Virginia 22314 |
| 1 copy | Dean<br>School of Medicine<br>Uniformed Services University of the<br>Health Sciences<br>4301 Jones Bridge Road<br>Bethesda, Maryland 20014 |
| 1 copy | Superintendent<br>Academy of Health Sciences, US Army<br>ATTN: AHS-COM<br>Fort Sam Houston, Texas 78234 |